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(54) Title: PORCINE CIRCOVIRUS RECOMBINANT POXVIRUS VACCINE

(57) Abstract: What is described is a recombinant poxvirus, such as avipox virus, containing foreign DNA from porcine circovirus 2. What are also described are immunological compositions containing the recombinant poxvirus for inducing an immunological response in a host animal to which the immunological composition is administered. Also described are methods of treating or preventing disease caused by porcine circovirus 2 by administering the immunological compositions of the invention to an animal in need of treatment or susceptible to infection by porcine circovirus 2.

TITLE OF THE INVENTION

Porcine Circovirus Recombinant Poxvirus Vaccine

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. application Serial No. 60/138,478, filed June 10, 1999 and from the U.S. utility application filed May 31, 2000. Reference is made to WO-A-99 18214, 1998, French applications Nos. 97/12382, 98/00873, 98/03707, filed October 3, 1997, January 22, 1998, and March 20, 1998, and WO99/29717. Each of the aforementioned U.S., PCT and French applications, and each document cited in the text and the record or prosecution of each of the aforementioned U.S., PCT and French applications ("application cited documents") and each document referenced or cited in each of the application cited documents, is hereby incorporated herein by reference; and, technology in each of the aforementioned U.S., PCT and French applications, and each document cited in the text and the record or prosecution of each of the aforementioned U.S., PCT and French applications can be used in the practice of this invention.

Several publications are referenced in this application. Full citation to these documents is found at the end of the specification preceding the claims, and/or where the document is cited. These documents pertain to the field of this invention; and, each of the documents cited or referenced in this application ("herein cited documents") and each document cited or referenced in herein cited documents are hereby incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to vectors, such as recombinant vectors; for instance, recombinant viruses, such as poxviruses, e.g., modified poxviruses and to methods of making and using the same. In some embodiments, the invention relates to recombinant avipox viruses, such as canarypox viruses, e.g., ALVAC. The invention further relates to such vectors, e.g., poxviruses, that express gene products, e.g., antigen(s), ORF(s), and/or epitope(s) of interest therefrom, of porcine circovirus 2 (PCV2); to immunological compositions or vaccines. The invention yet further relates to such vectors, e.g., poxviruses, that induce an immune response directed to or against PCV2 gene products and/or PCV2; and, to advantageously, such compositions that are immunological, immunogenic or vaccine compositions and/or confer protective immunity against infection by PCV2. The invention yet further relates to

the uses of and methods for making and using such vectors and compositions, as well as intermediates thereof, and said intermediates. And, the invention relates to the products therefrom, e.g., from the uses and methods involving the inventive recombinant or poxvirus, such as antibodies from expression.

BACKGROUND OF THE INVENTION

Postweaning multisystemic wasting syndrome (PMWS) is a recently recognized disease of young pigs. PMWS is characterized clinically by progressive weight loss and other symptoms such as tachypnea, dyspnea and jaundice. Pathologically, lymphocytic and granulomatous infiltrates, lymphadenopathy, and, more rarely, lymphocytic and granulomatous hepatitis and nephritis have been observed (Clark, 1997; Harding, 1997).

This disease has been described in different European countries as well as in North America. Treatment and prevention of this disease are not currently available.

Several lines of evidence point to porcine circovirus as the etiologic agent of PMWS (Ellis et al., 1998). Circoviruses have been recovered from pigs with PMWS, and antibodies to porcine circovirus have been demonstrated in pigs with the disease.

Circoviruses are single stranded circular DNA viruses found in a range of animal and plant species. Porcine circovirus was originally isolated as a contaminant from a continuous pig kidney cell line. The cell culture isolate has been designated PK-15 (Meehan et al., 1997). More recently, porcine circovirus obtained from pigs with PMWS has been compared to PK-15. Such viruses differ substantially from PK-15 at the nucleotide and protein sequence level, and have been designated PCV2 (Meehan et al., 1998; Hamel et al., 1998).

As many as thirteen open reading frames (ORFs) have been identified in the PCV2 genome (COL1 to COL13 in the French patent application 98 03707). Four of these ORFs share substantial homology with analogous ORFs within the genome of PK-15. ORF1 (Meehan et al., 1998; corresponding to COL4 in the French patent application 98 03707), comprising nt 398-1342 (GenBank accession number AF055392), has the potential to encode a protein with a predicted molecular weight of 37.7 kD. ORF2 (Meehan et al., 1998; corresponding to COL13 in the French patent application 98 03707), comprising nt 1381-1768 joined to 1-314 (GenBank accession number AF055392), may encode a protein with a predicted molecular weight of 27.8 kD. ORF3 (Meehan et al., 1998; corresponding to COL7 in the French patent

application 98 03707), comprising nt 1018-704 (GenBank accession number AF055392), may encode a protein with a predicted molecular weight of 11.9 kD. ORF4 (Meehan et al., 1998; corresponding to COL10 in the French patent application 98 03707), comprising nt 912-733 (GenBank accession number AF055392), may encode a protein with a predicted molecular weight of 6.5 kD.

ORF1 of PCV2 is highly homologous (86% identity) to the ORF1 of the PK-15 isolate (Meehan et al., 1998). The ORF1 protein of PK-15 has been partially characterized (Meehan et al., 1997 ; Mankertz et al., 1998a). It is known to be essential for virus replication, and is probably involved in the viral DNA replication.

Protein sequence identity between the respective ORF2s was lower (66% identity) than that of the ORF1s but each of the ORF2s shared a highly conserved basic N-terminal region, similar to that observed in the N-terminal region of the major structural protein of the avian circovirus chicken anemia virus (CAV) (Meehan et al., 1998). Recently, Mankertz et al. (1998b) has suggested that the ORF2 of the PK-15 isolate (designated ORF 1 in Mankertz et al., 1998b) codes for a capsid protein.

Greater differences were observed between the respective ORF3s and ORF4s of the PK-15 isolate and PCV2. In each case, there was a deletion of the C-terminal region of PCV2 ORF4 and ORF3 compared to the corresponding ORFs present in the genome of the PK-15 isolate. The highest protein sequence homology was observed at the N-terminal regions of both ORF3 and ORF4 (Meehan et al., 1998).

The transcription analysis of the genome of PCV2 has not been published yet. Recent data obtained with the PK-15 isolate indicated that the ORF2 transcript is spliced (Mankertz et al., 1998b).

Vaccinia virus has been used successfully to immunize against smallpox, culminating in the worldwide eradication of smallpox in 1980. With the eradication of smallpox, a new role for poxviruses became important, that of a genetically engineered vector for the expression of foreign genes (Panicali and Paoletti, 1982; Paoletti et al., 1984). Genes encoding heterologous antigens have been expressed in vaccinia, often resulting in protective immunity against challenge by the corresponding pathogen (reviewed in Tartaglia et al., 1990). A highly attenuated strain of vaccines, designated MVA, has also been used as a vector for poxvirus-based vaccines. Use of MVA is described in U.S. Patent No. 5,185,146.

Two additional vaccine vector systems involve the use of naturally host-restricted poxviruses, avipox viruses. Both fowlpoxvirus (FPV; Taylor et al. 1988a, b) and canarypoxvirus (CPV; Taylor et al., 1991 & 1992) have been engineered to express foreign gene products. Fowlpox virus (FPV) is the prototypic virus of the Avipox genus of the Poxvirus family. The virus causes an economically important disease of poultry which has been well controlled since the 1920's by the use of live attenuated vaccines. Replication of the avipox viruses is limited to avian species (Matthews, 1982) and there are no reports in the literature of avipoxvirus causing a productive infection in any non-avian species including man. This host restriction provides an inherent safety barrier to transmission of the virus to other species and makes use of avipoxvirus based vaccine vectors in veterinary and human applications an attractive proposition.

FPV has been used advantageously as a vector expressing antigens from poultry pathogens. The hemagglutinin protein of a virulent avian influenza virus was expressed in an FPV recombinant (Taylor et al., 1988c). After inoculation of the recombinant into chickens and turkeys, an immune response was induced which was protective against either a homologous or a heterologous virulent influenza virus challenge (Taylor et al., 1988c). FPV recombinants expressing the surface glycoproteins of Newcastle Disease Virus have also been developed (Taylor et al., 1990 ; Edbauer et al., 1990).

Other attenuated poxvirus vectors have been prepared by genetic modifications of wild type strains of virus. The NYVAC vector, derived by deletion of specific virulence and host-range genes from the Copenhagen strain of vaccinia (Tartaglia et al., 1992) has proven useful as a recombinant vector in eliciting a protective immune response against an expressed foreign antigen.

Another engineered poxvirus vector is ALVAC, derived from canarypox virus. ALVAC does not productively replicate in non-avian hosts, a characteristic thought to improve its safety profile (Taylor et al., 1991 & 1992). Both ALVAC and NYVAC are BSL-1 vectors.

One approach to the development of a subunit PCV2 vaccine is the use of live viral vectors to express relevant PCV2 ORFs. Recombinant poxviruses can be constructed in two steps known in the art and analogous to the methods for creating synthetic recombinants of poxviruses such as the vaccinia virus and avipox virus

described in U.S. Patent Nos. 4,769,330; 4,722,848; 4,603,112; 5,110,587; 5,174,993; 5,494,807; and 5,505,941, the disclosures of which are incorporated herein by reference. It can thus be appreciated that provision of a PCV2 recombinant poxvirus, and of compositions and products therefrom particularly ALVAC based PCV2 recombinants and compositions and products therefrom, especially such recombinants containing ORFs 1 and/or 2 of PCV2, and compositions and products therefrom would be a highly desirable advance over the current state of technology.

OBJECTS AND SUMMARY OF THE INVENTION

It is therefore an object of this invention to provide compositions and methods for treatment and prophylaxis of infection with PCV2. It is also an object to provide a means to treat or prevent PMWS.

In one aspect, the present invention relates to an antigenic, immunological, immunogenic, or vaccine composition or a therapeutic composition for inducing an antigenic, immunogenic or immunological response in a host animal inoculated with the composition. The composition advantageously includes a carrier or diluent and a recombinant virus, such as a recombinant poxvirus. The recombinant virus or poxvirus contains and expresses an exogenous nucleic acid molecule encoding an ORF, antigen, immunogen, or epitope of interest from PCV2, or a protein that elicits an immunological response against PCV2 or conditions caused by PCV2, such as PMWS. For instance, the recombinant virus can be a modified recombinant virus or poxvirus; for example, such a virus or poxvirus that has inactivated therein virus-encoded genetic functions, e.g., nonessential virus-encoded genetic functions, so that the recombinant virus has attenuated virulence and enhanced safety. And, the invention further provides the viruses used in the composition, as well as methods for making and uses of the composition and virus.

The virus used in the composition according to the present invention is advantageously a poxvirus, particularly a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus and more advantageously, ALVAC. The modified recombinant virus can include, e.g., within a non-essential region of the virus genome, a heterologous DNA sequence which encodes an antigenic protein, e.g., derived from PCV2 ORFs, e.g., PCV2 ORF 1 and/or 2.

In yet another aspect, the present invention relates to an immunogenic composition containing a modified recombinant virus having inactivated nonessential

virus-encoded genetic functions so that the recombinant virus has attenuated virulence and enhanced safety. The modified recombinant virus includes, e.g., within a non-essential region of the virus genome, a heterologous DNA sequence which encodes an antigenic protein (e.g., derived from PCV2 ORFs, especially ORFS 1 and/or 2) wherein the composition, when administered to a host, is capable of inducing an immunological response specific to the antigen.

In a still further aspect, the present invention relates to a modified recombinant virus having nonessential virus-encoded genetic functions inactivated therein so that the virus has attenuated virulence, and wherein the modified recombinant virus further contains DNA from a heterologous source, e.g., in a nonessential region of the virus genome. The DNA can code for PCV2 genes such as any or all of PCV2 ORF1, ORF2, ORF3, or ORF4 (Meehan et al., 1998), or epitope(s) of interest therefrom. The genetic functions can be inactivated by deleting an open reading frame encoding a virulence factor or by utilizing naturally host-restricted viruses. The virus used according to the present invention is advantageously a poxvirus, e.g., a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus.

Advantageously, the open reading frame that is deleted from the poxvirus or virus geneome is selected from the group consisting of J2R, B13R + B14R, A26L, A56R, C7L – K1L, and I4L (by the terminology reported in Goebel et al., 1990); and, the combination thereof. In this respect, the open reading frame comprises a thymidine kinase gene, a hemorrhagic region, an A type inclusion body region, a hemagglutinin gene, a host range gene region or a large subunit, ribonucleotide reductase; or, the combination thereof.

A suitable modified Copenhagen strain of vaccinia virus is identified as NYVAC (Tartaglia et al., 1992), or a vaccinia virus from which has been deleted J2R, B13R+B14R, A26L, A56R, C7L-K11 and I4L or a thymidine kinase gene, a hemorrhagic region, an A type inclusion body region, a hemagglutinin gene, a host range region, and a large subunit, ribonucleotide reductase (*See also* U.S. Patent No. 5,364,773, 5,494,807, and 5,762,938, with respect to NYVAC and vectors having additional deletions or inactivations from those of NYVAC that are also useful in the practice of this invention).

Preferably, the poxvirus vector is an ALVAC or, a canarypox virus which was attenuated, for instance, through more than 200 serial passages on chick embryo

fibroblasts (Rentschler vaccine strain), a master seed therefrom was subjected to four successive plague purifications under agar from which a plague clone was amplified through five additional passages. (*See also* U.S. Patent Nos. 5,756,103 and 5,766,599 with respect to ALVAC and TROVAC (an attenuated fowlpox virus useful in the practice of this invention); and U.S. Patents Nos. 6,004,777, 5,990,091, 5,770,212, 6,033,904, 5,869,312, 5,382,425, and WO 95/30018, with respect to vectors that also can be used in the practice of this invention, such as vectors having enhanced expression, vectors having functions deleted therefrom and vectors useful with respect to porcine hosts (for instance, vectors useful with porcine hosts can include a poxvirus, including a vaccinia virus, an avipox virus, a canarypox virus, and a swinepox virus), as well as with respect to terms used and teachings herein such as "immunogenic composition", "immunological composition", "vaccine", and "epitope of interest", and dosages, routes of administration, formulations, adjuvants, and uses for recombinant viruses and expression products therefrom).

The invention in yet a further aspect relates to the product of expression of the inventive recombinant poxvirus and uses therefor, such as to form antigenic, immunological or vaccine compositions for treatment, prevention, diagnosis or testing; and, to DNA from the recombinant poxvirus which is useful in constructing DNA probes and PCR primers.

These and other embodiments are disclosed or are obvious from and encompassed by the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

A better understanding of the present invention will be had by referring to the accompanying drawings, incorporated herein by reference, in which:

- FIG. 1 (SEQ ID NO:1) shows the nucleotide sequence of a 3.7 kilobase pair fragment of ALVAC DNA containing the C6 open reading frame.
- FIG. 2 shows the map of pJP102 donor plasmid.
- FIG. 3 (SEQ ID NO:8) shows the nucleotide sequence of the 2.5 kilobase pair fragment from pJP102 donor plasmid from the *Kpn*I (position 653) to the *Sac*I (position 3166) restriction sites.
- FIG. 4 shows the map of pJP105 donor plasmid.
- FIG. 5 shows the map of pJP107 donor plasmid.

- FIG. 6 (SEQ ID NO:11) shows the nucleotide sequence of the 3.6 kilobase pair fragment from pJP107 donor plasmid from the *Kpn*I (position 653) to the *Sac*I (position 4255) restriction sites.

DETAILED DESCRIPTION

In one aspect, the present invention relates to a recombinant virus, such as a recombinant poxvirus, containing therein a DNA sequence from PCV2, e.g., in a non-essential region of the poxvirus genome. The poxvirus is advantageously an avipox virus, such as fowlpox virus, especially an attenuated fowlpox virus, or a canarypox virus, especially an attenuated canarypox virus, such as ALVAC.

According to the present invention, the recombinant poxvirus expresses gene products of the foreign PCV2 gene. Specific ORFs of PCV2 are inserted into the poxvirus vector, and the resulting recombinant poxvirus is used to infect an animal. Expression in the animal of PCV2 gene products results in an immune response in the animal to PCV2. Thus, the recombinant poxvirus of the present invention may be used in an immunological composition or vaccine to provide a means to induce an immune response which may, but need not be, protective.

The administration procedure for recombinant poxvirus-PCV2 or expression product thereof, compositions of the invention such as immunological, antigenic or vaccine compositions or therapeutic compositions, can be via a parenteral route (intradermal, intramuscular or subcutaneous). Such an administration enables a systemic immune response, or humoral or cell-mediated responses.

More generally, the inventive poxvirus- PCV2 recombinants, antigenic, immunological or vaccine poxvirus- PCV2 compositions or therapeutic compositions can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts taking into consideration such factors as the age, sex, weight, species and condition of the particular patient, and the route of administration. The compositions can be administered alone, or can be co-administered or sequentially administered with compositions, e.g., with "other" immunological, antigenic or vaccine or therapeutic compositions thereby providing multivalent or "cocktail" or combination compositions of the invention and methods employing them. Again, the ingredients and manner (sequential or co-administration) of administration, as well as dosages can

be determined taking into consideration such factors as the age, sex, weight, species and condition of the particular patient, and, the route of administration. In this regard, reference is made to U.S. Patent No. 5,843,456, incorporated herein by reference, and directed to rabies compositions and combination compositions and uses thereof.

Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, vaginal, peroral, intragastric, etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. In such compositions the recombinant poxvirus or antigens may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, adjuvants, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation. Suitable dosages can also be based upon the Examples below.

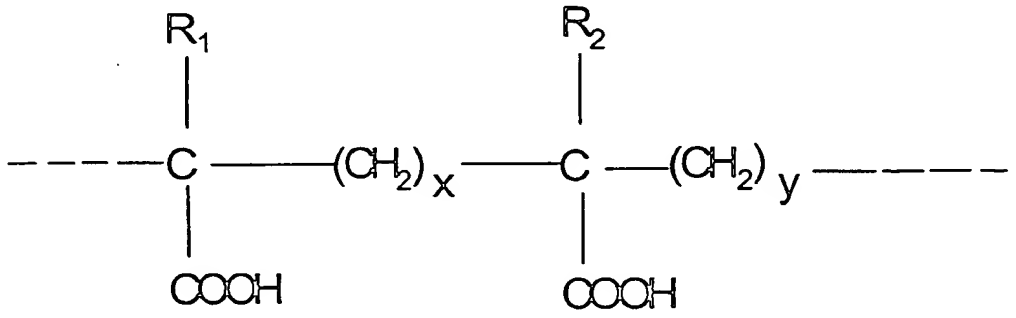
The compositions can contain at least one adjuvant compound chosen from the polymers of acrylic or methacrylic acid and the copolymers of maleic anhydride and alkenyl derivative.

The preferred adjuvant compounds are the polymers of acrylic or methacrylic acid which are cross-linked, especially with polyalkenyl ethers of sugars or polyalcohols. These compounds are known by the term carbomer (Phameuropa Vol. 8, No. 2, June 1996). Persons skilled in the art can also refer to U.S. Patent No. 2,909,462 (incorporated herein by reference) which describes such acrylic polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups, preferably not more than 8, the hydrogen atoms of at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing from 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under the name

Carbopol® (BF Goodrich, Ohio, USA) are particularly appropriate. They are cross-linked with an allyl sucrose or with allyl pentaerythritol. Among then, there may be mentioned Carbopol® 974P, 934P and 971P.

Among the copolymers of maleic anhydride and alkenyl derivative, the copolymers EMA® (Monsanto) which are copolymers of maleic anhydride and ethylene, linear or cross-linked, for example cross-linked with divinyl ether, are preferred. Reference may be made to J. Fields et al., Nature, **186** : 778-780, 4 June 1960, incorporated herein by reference.

From the point of view of their structure, the polymers of acrylic or methacrylic acid and the copolymers EMA® are preferably formed of basic units of the following formula :



in which :

- R_1 and R_2, which are identical or different, represent H or CH_3
- x = 0 or 1, preferably x = 1
- y = 1 or 2, with x + y = 2

For the copolymers EMA®, x = 0 and y = 2. For the carbomers, x = y =1.

The dissolution of these polymers in water leads to an acid solution which will be neutralized, preferably to physiological pH, in order to give the adjuvant solution into which the vaccine itself will be incorporated. The carboxyl groups of the polymer are then partly in COO^- form.

Preferably, a solution of adjuvant according to the invention, especially of carbomer, is prepared in distilled water, preferably in the presence of sodium chloride, the solution obtained being at acidic pH. This stock solution is diluted by adding it to the desired quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with NaCl, preferably physiological saline (NaCL 9 g/l) all at once in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4), preferably with NaOH. This solution at physiological pH will be used as it is for mixing with the vaccine, which may be especially stored in freeze-dried, liquid or frozen form.

The polymer concentration in the final vaccine composition will be 0.01% to 2% w/v, more particularly 0.06 to 1% w/v, preferably 0.1 to 0.6% w/v.

The immunological compositions according to the invention may be associated to at least one live attenuated, inactivated, or sub-unit vaccine, or recombinant vaccine (e.g. poxvirus as vector or DNA plasmid) expressing at least one immunogen from another pig pathogen.

The invention encompasses vectors encoding and expressing equivalent nucleotide sequences, that is to say the sequences which change neither the functionality or the strain specificity (say strain of type 1 and strain of type 2) of the gene considered or those of the polypeptides encoded by this gene. The sequences differing through the degeneracy of the code are, of course, included.

The PCV-2 sequences used in the examples are derived from Meehan *et al.* (Strain Imp.1010 ; ORF1 nucleotides 398-1342; ORF2 nucleotides 1381-314; and correspond respectively to ORF4 and ORF13 in U.S. application Serial No. 09/161,092 of 25 September 1998 and to COL4 and COL13 in WO-A-9918214). Other PCV-2 strains and their sequences have been published in WO-A-9918214 and are called Imp1008, Imp999, Imp1011-48285 and Imp1011-48121, as well as in A.L. Hamel *et al.* J. Virol. June 1998, vol **72**, 6: 5262-5267 (GenBank AF027217) and in I. Morozov *et al.* J. Clinical Microb. Sept. 1998 vol. **36**, 9: 2535-2541, as well as GenBank AF086834, AF086835 and AF086836, and give access to equivalent ORF sequences. These sequences, or ORFs therefrom, or regions thereof encoding an antigen or epitope of interest can also be used in the practice of this invention.

The invention also encompasses the equivalent sequences to those used herein and in documents cited herein; for instance, sequences that are capable of hybridizing

to the nucleotide sequence under high stringency conditions (see, e.g., Sambrook et al. (1989). Among the equivalent sequences, there may also be mentioned the gene fragments conserving the immunogenicity of the complete sequence, e.g., an epitope of interest.

The homology of the whole genome between PCV types 1 and 2 is about 75%. For ORF1, it is about 86%, and for ORF2, about 66%. On the contrary, homologies between genomes and between ORFs within type 2 are generally above 95%.

Also, equivalent sequences useful in the practice of this present invention, for ORF1, are those sequences having an homology equal or greater than 88%, advantageously 90% or greater, preferably 92% or 95% or greater with ORF1 of strain Imp1010, and for ORF2, are those sequences having an homology equal or greater than 80%, advantageously 85% or greater, preferably 90% or 95% or greater with ORF2 of strain Imp1010.

ORF1 and ORF2 according to Meehan 1998 has the potential to encode proteins with predicted molecular weights of 37.7 kD and 27.8 kD respectively. ORF3 and ORF4 (according to Meehan et al. 1998, correspond to ORF7 and ORF10 respectively in WO-A-9918214) has the potential to encode proteins with predicted molecular weights of 11.9 and 6.5 kD respectively. The sequence of these ORFs is also available in Genbank AF 055392. They can also be incorporated in plasmids and be used in accordance with the invention alone or in combination, e.g. with ORF1 and/or ORF2.

The other ORFs 1-3 and 5, 6, 8-9, 11-12 disclosed in U.S. application Serial No. 09/161,092 of 25 September 1998 (COLs 1-3 and 5, 6, 8-9, 11-12 in WO-A-9918214), or region(s) thereof encoding an antigen or epitope of interest, may be used in the practice of this invention, e.g., alone or in combination or otherwise with each other or with the ORFs 1 and 2 or region(s) thereof encoding antigen(s) or epitope(s).

This invention also encompasses the use of equivalent sequences; for instance, from ORFs of various PCV-2 strains cited herein. For homology, one can determine that there are equivalent sequences which come from a PCV strain having an ORF2 and/or an ORF1 which have an homology as defined above with the corresponding ORF of strain 1010.

For ORF3 according to Meehan, an equivalent sequence has homology thereto that is advantageously, for instance, equal or greater than 80%, for example 85% or

greater, preferably 90% or 95% or greater with ORF3 of strain Imp1010. For ORF4 according to Meehan 1998, advantageously an equivalent sequence has homology that is equal or greater than 86%, advantageously 90% or greater, preferably than 95% or greater with ORF4 of strain Imp1010.

From the genomic nucleotide sequence, e.g. those disclosed in WO-A-99 18214, it is routine art to determine the ORFs using a standard software, such as MacVector®. Also, alignment of genomes with that of strain 1010 and comparison with strain 1010 ORFs allows the one skilled in the art to readily determine the ORFs of the genome of another strain (e.g. other strains disclosed in WO-A-99 18214 or in other herein cited documents).

Using software or making sequence alignment is not undue experimentation and provides direct access to equivalent ORFs or nucleic acid molecules.

Nucleotide sequence homology can be determined using the "Align" program of Myers and Miller, ("Optimal Alignments in Linear Space", CABIOS *4*, 11-17, 1988, incorporated herein by reference) and available at NCBI. Alternatively or additionally, the term "homology" or "identity", for instance, with respect to a nucleotide or amino acid sequence, can indicate a quantitative measure of homology between two sequences. The percent sequence homology can be calculated as $(N_{ref} - N_{dif})*100/N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence similarity of 75% with the sequence AATCAATC (N_{ref} = 8; N_{dif}=2).

Alternatively or additionally, "homology" or "identity" with respect to sequences can refer to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman, 1983 PNAS USA 80:726, incorporated herein by reference), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-assisted analysis and interpretation of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Intelligenetics ™ Suite, Intelligenetics Inc. CA).. When RNA sequences are said to be similar, or

have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence.

RNA sequences within the scope of the invention can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

Additionally or alternatively, amino acid sequence similarity or identity or homology can be determined using the BlastP program (Altschul *et al.*, Nucl. Acids Res. 25, 3389-3402, incorporated herein by reference) and available at NCBI. The following references (each incorporated herein by reference) provide algorithms for comparing the relative identity or homology of amino acid residues of two proteins, and additionally or alternatively with respect to the foregoing, the teachings in these references can be used for determining percent homology or identity: Needleman SB and Wunsch CD, "A general method applicable to the search for similarities in the amino acid sequences of two proteins," J. Mol. Biol. 48:444-453 (1970); Smith TF and Waterman MS, "Comparison of Bio-sequences," Advances in Applied Mathematics 2:482-489 (1981); Smith TF, Waterman MS and Sadler JR, "Statistical characterization of nucleic acid sequence functional domains," Nucleic Acids Res., 11:2205-2220 (1983); Feng DF and Dolittle RF, "Progressive sequence alignment as a prerequisite to correct phylogenetic trees," J. of Molec. Evol., 25:351-360 (1987); Higgins DG and Sharp PM, "Fast and sensitive multiple sequence alignment on a microcomputer," CABIOS, 5: 151-153 (1989); Thompson JD, Higgins DG and Gibson TJ, "ClusterW: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, positions-specific gap penalties and weight matrix choice, Nucleic Acid Res., 22:4673-480 (1994); and, Devereux J, Haeberlie P and Smithies O, "A comprehensive set of sequence analysis program for the VAX," Nucl. Acids Res., 12: 387-395 (1984).

This invention not only allows for administration to adult pigs, but also to the young and to gestating females; in the latter case, this makes it possible, in particular, to confer passive immunity onto the newborns (maternal antibodies). Preferably, female pigs are inoculated prior to breeding; and/or prior to serving, and/or during gestation. Advantageously, at least one inoculation is done before serving and it is preferably followed by an inoculation to be performed during gestation, e.g., at about mid-gestation (at about 6-8 weeks of gestation) and/or at the end of gestation (at about

11-13 weeks of gestation). Thus, an advantageous regimen is an inoculation before mating and/o serving and a booster inoculation during gestation. Thereafter, there can be reinoculation before each serving and/or during gestation at about mid-gestation and/or at the end of gestation. Preferably, reinoculations are during gestation. Male pigs also can be inoculated, e.g., prior to mating.

Piglets, such as piglets from vaccinated females (e.g., inoculated as herein discussed), are inoculated within the first weeks of life, e.g., inoculation at one and/or two and/or three and/or four and/or five weeks of life. Preferably, piglets are first inoculated within the first week of life or within the third week of life (e.g., at the time of weaning). Advantageously, such piglets are then boosted two to four weeks later.

The present invention is additionally described by the following illustrative, non-limiting Examples.

EXAMPLES

The invention in a preferred embodiment is directed to recombinant poxviruses containing therein a DNA sequence from PCV2 in a nonessential region of the poxvirus genome. The recombinant poxviruses express gene products of the foreign PCV2 gene. In particular, ORF2 and ORF1 genes encoding PCV2 proteins were isolated, characterized and inserted into ALVAC (canarypox vector) recombinants. The molecular biology techniques used are the ones described by Sambrook et al. (1989).

Cell Lines and Virus Strains. The strain of PCV2 designated Imp.1010-Stoon has been previously described (Meehan et al., 1998). It was isolated from mesenteric lymph node tissues from a diseased pig originating from Canada. Cloning of the PCV2 genome was described by Meehan et al. (1998). Plasmid pGem7Z-Imp1010-Stoon-EcoRI No. 14 contains the PCV2 genome as an *Eco*RI fragment inserted into the *Eco*RI site of plasmid pGem-7Z (Promega, Madison, WI). The complete nucleotide sequence of the Imp.1010-Stoon PCV2 strain has been determined by Meehan et al. (1998) and is available under the GenBank accession number AF055392.

The parental canarypox virus (Rentschler strain) is a vaccinal strain for canaries. The vaccine strain was obtained from a wild type isolate and attenuated through more than 200 serial passages on chick embryo fibroblasts. A master viral seed was subjected to four successive plaque purifications under agar and one plaque

clone was amplified through five additional passages after which the stock virus was used as the parental virus in *in vitro* recombination tests. The plaque purified canarypox isolate is designated ALVAC. ALVAC was deposited November 14, 1996 under the terms of the Budapest Treaty at the American Type Culture Collection, ATCC accession number VR-2547.

The generation of poxvirus recombinants involves different steps: (1) construction of an insertion plasmid containing sequences ("arms") flanking the insertion locus within the poxvirus genome, and multiple cloning site (MCS) localized between the two flanking arms (e.g., see Example 1); (2) construction of donor plasmids consisting of an insertion plasmid into the MCS of which a foreign gene expression cassette has been inserted (e.g. see Examples 2 to 5); (3) *in vitro* recombination in cell culture between the arms of the donor plasmid and the genome of the parental poxvirus allowing the insertion of the foreign gene expression cassette into the appropriate locus of the poxvirus genome, and plaque purification of the recombinant virus (e.g. see Example 6).

PCV2 recombinant immunogens may be used in association with PCV1 immunogens, for immunization of animals against PMWS. In a least preferred approach, PCV1 immunogens may be used without PCV2 immunogens.

Example 1 - **CONSTRUCTION OF CANARYPOX INSERTION PLASMID AT C6 LOCUS**

Figure 1 (SEQ ID NO:1) is the sequence of a 3.7 kb segment of canarypox DNA. Analysis of the sequence revealed an ORF designated C6L initiated at position 377 and terminated at position 2254. The following describes a C6 insertion plasmid constructed by deleting the C6 ORF and replacing it with a multiple cloning site (MCS) flanked by transcriptional and translational termination signals. A 380 bp PCR fragment was amplified from genomic canarypox DNA using oligonucleotide primers C6A1 (SEQ ID NO:2) and C6B1 (SEQ ID NO:3). A 1155 bp PCR fragment was amplified from genomic canarypox DNA using oligonucleotide primers C6C1 (SEQ ID NO:4) and C6D1 (SEQ ID NO:5). The 380 bp and 1155 bp fragments were fused together by adding them together as template and amplifying a 1613 bp PCR fragment using oligonucleotide primers C6A1 (SEQ ID NO:2) and C6D1 (SEQ ID NO:5). This fragment was digested with *Sac*I and *Kpn*I, and ligated into pBluescript SK+ (Stratagene, La Jolla, CA, USA) digested with *Sac*I/*Kpn*I. The resulting plasmid,

pC6L was confirmed by DNA sequence analysis. It consists of 370 bp of canarypox DNA upstream of C6 ("C6 left arm"), vaccinia early termination signal, translation stop codons in six reading frames, an MCS containing *Sma*I, *Pst*I, *Xho*I and *Eco*RI sites, vaccinia early termination signal, translation stop codons in six reading frames and 1156 bp of downstream canary pox sequence ("C6 right arm").

Plasmid pJP099 was derived from pC6L by ligating a cassette containing the vaccinia H6 promoter (described in Taylor et al. (1988c), Guo et al. (1989), and Perkus et al. (1989)) coupled to a foreign gene into the *Sma*I/*Eco*RI sites of pC6L. This plasmid pJP099 contains a unique *Eco*RV site and a unique *Nru*I site located at the 3' end of the H6 promoter, and a unique *Sal*I site located between the STOP codon of the foreign gene and the C6 left arm. The ~4.5 kb *Eco*RV/*Sal*I or *Nru*I/*Sal*I fragment from pJP099 contains therefore the plasmid sequence (pBluescript SK+ ; Stratagene, La Jolla, CA, USA), the 2 C6 arms and the 5' end of the H6 promoter until the *Eco*RV or *Nru*I site.

Sequences of the primers:

Primer C6A1 (SEQ ID NO:2)

ATCATCGAGCTCGCGGCCGCCTATCAAAAGTCTTAATGAGTT

Primer C6B1 (SEQ ID NO:3)

GAATTCCTCGAGCTGCAGCCCGGGTTTTTATAGCTAATTAGTCATTTTTTC GTAAGTAAGTATTTTTATTTAA

Primer C6C1 (SEQ ID NO:4)

CCCGGGCTGCAGCTCGAGGAATTCTTTTTATTGATTAACTAGTCAAATGAG TATATATAATTGAAAAAGTAA

Primer C6D1 (SEQ ID NO:5)

GATGATGGTACCTTCATAAATACAAGTTTGATTAAACTTAAGTTG

Example 2 - CONSTRUCTION OF ALVAC DONOR PLASMID FOR PCV2 ORF2

Plasmid pGem7Z-Imp1010-Stoon-EcoRI No. 14, containing the PCV2 genome as an *Eco*RI fragment in plasmid pGem-7Z, was digested with *Eco*RI, and a 1768bp fragment was isolated and ligated.

In order to insert PCV2 ORF 2 into an appropriate ALVAC insertion vector: Primers JP760 (SEQ ID NO:6) and JP773 (SEQ ID NO:7) were used to amplify PCV2 ORF 2 from the 1768bp ligated *Eco*RI fragment (see above) resulting in PCR

J1304. Primer JP760 (SEQ ID NO:6) contains the 3' end of the H6 promoter from *Eco*RV and the 5' end of PCV2 ORF 2. Primer JP773 (SEQ ID NO:7) contains the 3' end of PCV2 ORF 2 followed by a *Sal*I site. The product of PCR J1304 was then digested with *Eco*RV/*Sal*I and cloned as a ~750 bp fragment into a ~4.5 kb *Eco*RV/*Sal*I fragment from pJP099 (see above in Example 1). The resulting plasmid was confirmed by sequence analysis and designated pJP102 (see the map of pJP102 in Figure 2 and the sequence (SEQ ID NO:8) in Figure 3). The sequence of ORF 2 matches sequence available in GenBank, Accession Number AF055392. The donor plasmid pJP102 (linearized with *Not*I) was used in an *in vitro* recombination (IVR) test to generate ALVAC recombinant vCP1614 (see Example 6).

Sequence of the primers:

JP760 (SEQ ID NO:6)

CAT-CAT-CAT-GAT-ATC-CGT-TAA-GTT-TGT-ATC-GTA-ATG-ACG-TAT-CCA-AGG-AGG-CG

JP773 (SEQ ID NO:7)

TAC-TAC-TAC-GTC-GAC-TTA-GGG-TTT-AAG-TGG-GGG-GTC

Example 3 - CONSTRUCTION OF AN ALVAC DONOR PLASMID FOR PCV2 ORF2 AND ORF1

PCV2 ORF 1 was amplified by PCR using primers JP774 (SEQ ID NO:9) and JP775 (SEQ ID NO:10) on plasmid pGem7Z-Imp1010-Stoon-EcoRI No. 14 resulting in PCR J1311. Primer JP774 (SEQ ID NO:9) contains the 3' end of the H6 promoter from *Nru*I and the 5' end of PCV2 ORF1. Primer JP775 (SEQ ID NO:10) contains the 3' end of PCV2 ORF1 followed by a *Sal*I site. The product of PCR J1311 (~1 Kb) was cloned into pCR2.1 (Invitrogen, Carlsbad, CA). The resulting plasmid was confirmed by sequence analysis and designated pJP104. The sequence of ORF1 matches sequence available in GenBank, Accession Number AF055392. A ~970 bp *NruI*/*Sal*I fragment was isolated from pJP104 and cloned into a ~4.5 kb *NruI*/*Sal*I fragment from pJP099 (see Example 1), resulting in a plasmid which was confirmed by restriction analysis and designated pJP105 (see Figure 4). The donor plasmid pJP105 could be used in an *in vitro* recombination test (described in Example 6) to generate ALVAC recombinant expressing the PCV2 ORF1.

A ~838bp *Bam*HI/*Sal*I from pJP102 (see Example 2) was blunted using the Klenow fragment of DNA polymerase, and was cloned into the Klenow-blunted

*Eco*RI site of pJP105. Clones were checked for orientation of insert by restriction analysis and a head-to-head orientation was chosen. This plasmid was confirmed by sequence analysis and designated pJP107 (see the map of pJP107 in Figure 5 and the sequence (SEQ ID NO:11) in Figure 6). The donor plasmid pJP107 (linearized with *Not*I) was used in an *in vitro* recombination 5 (IVR) test to generate the ALVAC recombinant vCP1615 (see Example 6).

Sequence of the primers:

JP774 (SEQ ID NO:9)

CAT-CAT-CAT-TCG-CGA-TAT-CCG-TTA-AGT-TTG-TAT-CGT-AAT-GCC-CAG-CAA-GAA-GAA-TGG

JP775 (SEQ ID NO:10)

TAC-TAC-TAC-GTC-GAC-TCA-GTA-ATT-TAT-TTC-ATA-TGG

Example 4 - CONSTRUCTION OF ALVAC DONOR PLASMID FOR PCV1 ORF2

Plasmid pPCV1 (B. Meehan *et al.* J. Gen. Virol. 1997. **78**. 221-227), containing the PCV1 genome as a *Pst*I fragment in plasmid pGem-7Z, was used as a template to amplify the PCV1 ORF2.

In order to insert PCV2 ORF 2 into an appropriate ALVAC insertion vector : Primers JP787 (SEQ ID NO:12) and JP788 (SEQ ID NO:13) were used to amplify PCV1 ORF 2 from plasmid pPCV1 (see above) resulting in PCR J1315. Primer JP787 (SEQ ID NO:12) contains the 3' end of the H6 promoter from *Eco*RV and ORF 2 followed by a *Sal*I site. The product of PCR J1315 was then digested with *Eco*RV/*Sal*I and cloned as a ~750 bp fragment into a ~4.5 kb *Eco*RV/*Sal*I fragment from pJP099 (see above in Example 1). The resulting plasmid was confirmed by sequence analysis and designated pJP113. The sequence of ORF 2 matches sequence available in GenBank, Accession Number U49186. The donor plasmid pJP113 (linearized with *Not*I) was used in an *in vitro* recombination (IVR) test to generate ALVAC recombinant vCP1621 (see Example 7).

Sequence of the primers:

JP787 (SEQ ID NO:12)

CAT-CAT-CAT-GAT-ATC-CGT-TAA-GTT-TGT-ATC-GTA-ATG-ACG-TGG-CCA-AGG-AGG-CG

JP788 (SEQ ID NO:13)

TAC-TAC-TAC-GTC-GAC-TTA-TTT-ATT-TAG-AGG-GTC-TTT-TAG-G

Example 5 - CONSTRUCTION OF AN ALVAC DONOR PLASMID FOR PCV1 ORF2 AND ORF1

Plasmid pPCV1 (see Example 4 above), containing the PCV1 genome as a *Pst*I fragment in plasmid pGem-7Z, was digested with *Pst*I, and a 1759 bp fragment was isolated and ligated.

Primers JP789 (SEQ ID NO:14) and JP790 (SEQ ID NO:15) were used to amplify PCV1 ORF1 from the 1759 bp ligated *Pst*I fragment (see above), resulting in PCR J1316. Primer JP789 (SEQ ID NO:14) contains the 3' end of the H6 promoter from *Nru*I and the 5' end of PCV1 ORF1. Primer JP790 (SEQ ID NO:15) contains the 3' end of PCV1 ORF1 followed by a *Sal*I site. The product of PCR J1316 (~1 Kb) was cloned into pCR2.1 (Invitrogen, Carlsbad, CA). The resulting plasmid was confirmed by sequence analysis and designated pJP114. The sequence of ORF1 matches sequence available in GenBank, Accession Number U49186. A ~970 bp *Nru*I/*Sal*I fragment was isolated from pJP114 and cloned into a ~4.5 kb *Nru*I/*Sal*I fragment from pJP099 (see Example 1), resulting in a plasmid which was confirmed by restriction analysis and designated pJP115. The donor plasmid pJP115 could be used in an *in vitro* recombination test (described in Example 7) to generate ALVAC recombinant expressing the PCV1 ORF1.

A ~838bp *Bam*HI/*Sal*I from pJP113 (see Example 4) was blunted using the Klenow fragment of DNA polymerase, and was cloned into the Klenow-blunted *Eco*RI site of pJP115. Clones were checked for orientation of insert by restriction analysis and a head-to-head orientation was chosen. This plasmid was confirmed by sequence analysis and designated pJP117. The donor plasmid pJP117 (linearized with *Not*I) was used in an *in vitro* recombination (IVR) test to generate the ALVAC recombinant vCP1622 (see Example 7).

Sequence of the primers:

JP789 (SEQ ID NO:14)

CAT-CAT-CAT-TCG-CGA-TAT-CCG-TTA-AGT-TTG-TAT-CGT-AAT-GCC-AAG-CAA-GAA-AAG-CGG

JP790 (SEQ ID NO:15)

TAC-TAC-TAC-GTC-GAC-TCA-GTA-ATT-TAT-TTT-ATA-TGG

Example 6 - GENERATION OF ALVAC-PCV2 RECOMBINANTS

Plasmids pJP102 (see Example 2 and Figure 2) and pJP107 (see Example 3 and Figure 5) were linearized with *Not*I and transfected into ALVAC infected primary CEF cells by using the calcium phosphate precipitation method previously described (Panicali and Paoletti, 1982 ; Piccini et al., 1987). Positive plaques were selected on the basis of hybridization to specific PCV2 radiolabeled probes and subjected to four sequential rounds of plaque purification until a pure population was achieved. One representative plaque from each IVR was then amplified and the resulting ALVAC recombinants were designated vCP1614 and vCP1615. The vCP1614 virus is the result of recombination events between ALVAC and the donor plasmid pJP102, and it contains the PCV2 ORF2 inserted into the ALVAC C6 locus. The vCP1615 virus is the result of recombination events between ALVAC and the donor plasmid pJP107, and it contains the PCV2 ORF2 and ORF1 inserted into the ALVAC C6 locus in a head-to-head orientation.

In a similar fashion, a recombinant ALVAC expressing only PCV2 ORF1 can be generated using the donor plasmid pJP105 described in Example 3.

Immunofluorescence. In order to determine if the PCV2 proteins were expressed in ALVAC recombinant infected Vero cells, immunofluorescence (IF) analysis was performed. Infected Vero cells were washed with PBS 24 hrs after infection (m.o.i. of approx. 10) and fixed with 95% cold aceton for 3 minutes at room temperature. Five monoclonal antibody (MAb) preparations (hybridoma supernatant) specific for PCV2 ORF1 (PCV2 199 1D3GA & PCV2 210 7G5GD) or ORF2 (PCV2 190 4C7CF, PCV2 190 2B1BC & PCV2 190 3A8BC) were used as the first antibody. These specific monoclonal antibodies were obtained from Merial-Lyon. Monoclonal antibodies can also be obtained following the teachings of documents cited herein, e.g. WO-A-99 18214, 1998, French applications Nos. 97/12382, 98/00873, 98/03707, filed October 3, 1997, January 22, 1998, and March 20, 1998, and WO99/29717, incorporated herein by reference. The IF reaction was performed as described by Taylor et al. (1990).

PCV2 specific immunofluorescence with the three ORF2-specific antibodies could be detected in cells infected with vCP1614 and cells infected with vCP1615. PCV2 specific immunofluorescence with the two ORF1-specific antibodies could be detected in cells infected with vCP1615 only. These results indicated that, as

expected, vCP1614 expresses only ORF2, whereas vCP1615 expresses both ORF1 and ORF2. No fluorescence was detected in parental ALVAC infected Vero cells, nor in uninfected Vero cells.

Example 7 - GENERATION OF ALVAC-PCV1 RECOMBINANTS

Plasmids pJP113 (see Example 4) and pJP117 (see Example 5) were linearized with *Not*I and transfected into ALVAC infected primary CEF cells by using the calcium phosphate precipitation method previously described (Panicali and Paoletti, 1982; Piccini et al., 1987). Positive plaques were selected on the basis of hybridization to specific PCV1 radiolabeled probes and subjected to four sequential rounds of plaque purification until a pure population was achieved. One representative plaque from each IVR was then amplified and the resulting ALVAC recombinants were designated vCP1621 and vCP1622. The vCP1621 virus is the result of recombination events between ALVAC and the donor plasmid pJP113, and it contains the PCV1 ORF2 inserted into the ALVAC C6 locus. The vCP1622 virus is the result of recombination events between ALVAC and the donor plasmid pJP117, and it contains the PCV1 ORF2 and ORF1 inserted into the ALVAC C6 locus in a head-to-head orientation.

In a similar fashion, a recombinant ALVAC expressing only PCV1 ORF1 can be generated using the donor plasmid pJP115 described in Example 5.

Immunofluorescence. In order to determine if the PCV1 proteins were expressed in ALVAC recombinant infected Vero cells, immunofluorescence (IF) analysis was performed. Infected Vero cells were washed with PBS 24 hrs after infection (m.o.i. of approx. 10) and fixed with 95% cold aceton efor 3 minutes at room temperature. A specific anti-PCV1 pig polyclonal serum (Allan G. *et al.* Vet. Microbiol. 1999. **66**: 115-123) was used as the first antibody. The IF reaction was performed as described by Taylor et al. (1990).

PCV1 specific immunofluorescence could be detected in cells infected with vCP1621 and cells infected with vCP1622. These results indicated that, as expected, vCP1621 and vCP1622 express PCV1-specific products. No fluorescence was detected with a PCV2-specific pig polyclonal serum in cells infected with vCP1621 and in cells infected with vCP1622. No fluorescence was detected in parental ALVAC infected Vero cells, nor in uninfected Vero cells.

Example 8 - FORMULATION OF RECOMBINANT CANARYPOX VIRUSES WITH CARBOPOL™ 974P

For the preparation of vaccines, recombinant canarypox viruses vCP1614 and vCP1615 (Example 6) can be mixed with solutions of carbomer. In the same fashion, recombinant canarypox viruses vCP1621 and vCP1622 (Example 7) can be mixed with solutions of carbomer. The carbomer component used for vaccination of pigs according to the present invention is the Carbopol™ 974P manufactured by the company BF Goodrich (molecular weight of # 3,000,000). A 1.5 % Carbopol™ 974P stock solution is first prepared in distilled water containing 1 g/l of sodium chloride. This stock solution is then used for manufacturing a 4 mg/ml Carbopol™ 974P solution in physiological water. The stock solution is mixed with the required volume of physiological water, either in one step or in several successive steps, adjusting the pH value at each step with a 1N (or more concentrated) sodium hydroxide solution to get a final pH value of 7.3-7.4. This final Carbopol™ 974P solution is a ready-to-use solution for reconstituting a lyophilized recombinant virus or for diluting a concentrated recombinant virus stock. For example, to get a final viral suspension containing 10^8 pfu per dose of 2 ml, one can dilute 0,1 ml of a 10^9 pfu/ml stock solution into 1,9 ml of the above Carbopol™ 974P 4 mg/ml ready-to-use solution. In the same fashion, Carbopol™ 974P 2 mg/ml ready-to-use solutions can also be prepared.

Example 9 - IMMUNIZATION OF PIGS AND SUBSEQUENT CHALLENGE

9.1. IMMUNIZATION OF 1 DAY-OLD PIGLETS

Groups of piglets, caesarian-derived at Day 0, are placed into isolators. The piglets are vaccinated by intramuscular route at Day 2 with various vaccine solutions. Vaccine viral suspensions are prepared by dilution of recombinant viruses stocks in sterile physiological water (NaCl 0.9 %). Suitable ranges for viral suspensions can be determined empiracally, but will generally range from 10^6 to 10^{10}, and preferably about 10^{10} , pfu/dose. Vaccine solutions can also be prepared by mixing the recombinant virus suspension with a solution of Carbopol™ 974P, as described in Example 8.

Piglets are vaccinated either with:

Recombinant virus vCP1614 (Example 2);

Recombinant virus vCP1615 (Example 3);

Recombinant virus vCP1614 mixed with Carbopol (4 mg/ml solution); or

Recombinant virus vCP1615 mixed with Carbopol (4 mg/ml solution).

The viral suspensions contain 10^8 plaque forming units (pfu) per dose. Each viral suspension is injected by intramuscular route under a volume of 1 ml. The intramuscular injection is administered into the muscles of the neck.

Two injections of viral suspensions are administered at Day 2 and Day 14 of the experiment. A challenge is done on Day 21 by an oronasal administration of a viral suspension prepared from a culture of PCV-2 virulent strain. After challenge, piglets are monitored during 3 weeks for clinical signs specific of the post-weaning multisystemic syndrome. The following signs are scored :

Rectal temperature: daily monitoring for 2 weeks post-challenge, then 2 measures of rectal temperature during the third week.

Weight: piglets are weighed right before the challenge, and then weekly during the first 3 weeks post-challenge.

Blood samples are taken at Day 2, day 14, Day 21, Day 28, Day 35 and Day 42 of the experiment in order to monitor viremia levels and anti-PCV-2 specific antibody titers.

Necropsies: at Day 42, all surviving piglets are humanely euthanized and necropsied to look for specific PWMS macroscopic lesions. Tissue samples are prepared from liver, lymph nodes, spleen, kidneys and thymus in order to look for specific histological lesions.

9.2. IMMUNIZATION OF 5-7 WEEK-OLD PIGLETS

5-7 week-old piglets, free of anti-PCV-2 specific maternal antibodies, are vaccinated by intramuscular route with various vaccine solutions. Vaccine viral suspensions are prepared by dilution of recombinant viruses stocks in sterile physiological water (NaCl 0.9 %). Vaccine solutions can also be prepared by mixing the recombinant virus suspension with a solution of Carbopol™ 974P, as described in Example 8.

Piglets are vaccinated either with:

Recombinant virus vCP1614 (Example 2);

Recombinant virus vCP1615 (Example 3);

Recombinant virus vCP1614 mixed with Carbopol (4 mg/ml solution); or

Recombinant virus vCP1615 mixed with Carbopol (4 mg/ml solution).

The viral suspensions contain 10^8 plaque forming units (pfu) per dose. Each viral suspension is injected by intramuscular route under a volume of 2 ml. The intramuscular injection is administered into the muscles of the neck.

Two injections of the viral suspensions are administered at Day 0 and Day 21 of the experiment. A challenge is done at Day 35 by an oronasal administration of a viral suspension prepared from a culture of PCV-2 virulent strain. After challenge, piglets are monitored during 8 weeks for clinical signs specific of the post-weaning multisystemic syndrome. The clinical monitoring is identical to the one described in Example 9.1. except that total duration of monitoring is 8 weeks instead of 3 weeks.

Necropsies are done throughout the experiment for piglets dying from the challenge and at the end of the experiment (Day 97) for all surviving piglets. Tissue samples are the same as described in Example 9.1.

9.3. IMMUNIZATION OF NEWBORN PIGLETS

Groups of 3 or 4 piglets, caesarian-delivered day 0are placed into isolators. Day 2 the piglets are vaccinated with 10^8 pfu of vCP1614, vCP1615 or parental ALVAC vector in 1 ml of PBS by intramuscular route on the side of the neck. A second injection of vaccine or placebo is administered at day 14. Vaccination with ALVAC recombinant is well tolerated by piglets and no evidence of adverse reaction to vaccination is noted. The piglets are challenged day 21 by oronasal administration of a PCV-2 viral suspension, 1 ml in each nostril. Day 45 necropsies are performed and samples of tissues are collected for virus isolation.

Necropsy results:

- PMWS is characterized generally by lymphadenopathy and more rarely by hepatitis or nephritis. So the gross findings in lymph nodes are scored for each piglet in the following manner : 0 = no visible enlargement of lymph nodes ; 1 = mild lymph nodes enlargement, restricted to bronchial lymph nodes ; 2 = moderate lymph nodes enlargement, restricted to bronchial lymph nodes ; 3 = severe lymph nodes enlargement, extended to bronchial, submandibullar prescapular and inguinal lymph nodes.

Groups	Scores
vCP 1614	0.5
	0.0
	0.0
	1.0
mean	0.38
standard deviation	0.48
vCP 1615	0.0
	0.5
	0.5
	1.0
mean	0.5
standard deviation	0.41
Controls	2.0
	2.5
	2.5
	2.5
mean	2.38
standard deviation	0.25

Bronchial lymphadenopathy for PCV-2 is a prominent gross finding. A significant reduction of the lymph nodes lesion in relation to control group is observed after immunization with vCP 1614 and vCP 1615 ($p \leq 0.05$).

* * *

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

REFERENCES

1. Clark, E.G. Proc. Amer. Assoc. Swine Pract., pp. 499-501 (1997).
2. Edbauer, C., R. Weinberg, J. Taylor, A. Rey-Senelonge, J.F. Bouquet, P. Desmettre and E. Paoletti, Virology 179, 901-904 (1990).
3. Ellis, J., L. Hassard, E. Clark, J. Harding, G. Allan, P. Willson, J. Strakappe, K. Martin, F. McNeilly, B. Meehan, D. Todd, D. Haines, Can. Vet. J. 39, 44-51 (1998).
4. Goebel, S.J., G.P. Johnson, M.E. Perkus, S.W. Davis, J.P. Winslow, E. Paoletti, Virology 179, 247-266, 517-563 (1990).
5. Guo, P., S. Goebel, S. Davis, M.E. Perkus, B. Languet, P. Desmettre, G. Allen, and E. Paoletti, J. Virol. 63, 4189-4198 (1989).
6. Hamel, A.L., L.L. Lin and G. P.S. Nayar, J. Virol. 72, 5262-5267 (1998).
7. Harding J.C., Proc. Am. Assoc. Swine Pract. 28, 503 (1997).
8. Mankertz, A., J. Mankertz, K. Wolf, H.-J. Buhk, Gen. Virol. 79, 381-384 (1998a).
9. Mankertz, J., H.-J. Buhk, G. Blaess, A. Mankertz, Virus Gene 16, 267-276 (1998b).
10. Matthews, R.E.F., Intervirology 17, 42-44 (1982).
11. Meehan, B.M., J.L. Creelan, M.S. McNulty, D. Todd, J. Gen. Virol. 78, 221-227 (1997).
12. Meehan, B.M., F. McNeilly, D. Todd, S. Kennedy, V.A. Jewhurst, J.A. Ellis, L.E. Hassard, E.G. Clark, D.M. Haines, G.M. Allan, J. Gen. Virol. 79, 2171-2179 (1998).
13. Nayar, G.P.S., A. Hamel and L. Lin. Can. Vet. J. 38, 385-386 (1997).
14. Panicali, D. and E. Paoletti, Proc. Natl. Acad. Sci. USA 79, 4927-4931 (1982).
15. Paoletti, E., B.R. Lipinskaks, C. Samsonoff, S. Mercer, and D. Panicali, Proc. Natl. Acad. Sci. U.S.A. 81, 193-197 (1984).
16. Perkus, M.E., K. Limbach, and E. Paoletti, J. Virol. 63, 3829-3836 (1989).
17. Piccini, A., M.E. Perkus, and E. Paoletti, In Methods in Enzymology, Vol. 153, eds. Wu, R., and Grossman, L., (Academic Press) pp. 545-563 (1987).
18. Sambrook, J., E.F. Fritsch, and T. Maniatis, In Molecular cloning: A laboratory manual, 2nd edition, (Cold Spring Harbor Press, NY) (1989).

19. Tartaglia, J., J. Winslow, S. Goebel, G.P. Johnson, J. Taylor, and E. Paoletti, J. Gen. Virol. 71, 1517-1524 (1990).

20. Tartaglia, J., Perkus ME, Taylor J, Norton EK, Audonnet JC, Cox WI, Davis SW, van der Hoeven J, Meignier B, Riviere M, and E. Paoletti, Virology **188**, 217-32 (1992).

21. Taylor, J., R. Weinberg, B. Languet, Ph. Desmettre and E. Paoletti, Vaccine **6**, 497-503 (1988a).

22. Taylor, J. and E. Paoletti, Vaccine **6**, 466-468 (1988b).

23. Taylor, J., R. Weinberg, Y. Kawaoka, R. Webster and E. Paoletti, Vaccine **6**, 504-508 (1988c).

24. Taylor, J., C. Edbauer, A. Rey-Senelonge, J.F. Bouquet, E. Norton, S. Goebel, P. Desmettre and E. Paoletti, J. Virol. **64**, 1441-1450 (1990).

25. Taylor, J., C. Trimarchi, R. Weinberg, B. Languet, F. Guillemin, P. Desmettre and E. Paoletti, Vaccine **9**, 190-193 (1991).

26. Taylor J, Weinberg R, Tartaglia J, Richardson C, Alkhatib G, Briedis D, Appel M, Norton E, Paoletti E., Virology**187**, 321-328 (1992).

27. Todd, D., F.D. Niagro, B.W. Ritchie, W. Curran, G.M. Allan, P.D. Lukert, K.S. Latimer, W.L. Steffens, M.S. McNulty, Arch. Virol. **117**, 129-135 (1991).

WHAT IS CLAIMED IS:

1. A recombinant virus comprising DNA from porcine circovirus 2.
2. The recombinant virus of claim 1 which is a poxvirus.
3. The recombinant poxvirus of claim 2 which is an avipox virus.
4. The recombinant avipox virus of claim 3 which is ALVAC.
5. The recombinant ALVAC virus of claim 4, wherein the DNA from porcine circovirus 2 codes for and is expressed as the porcine circovirus major capsid protein or an epitope of interest.
6. The recombinant ALVAC virus of claim 4, wherein the DNA from porcine circovirus 2 comprises of the open reading frame 2 (ORF2) of porcine circovirus 2.
7. The recombinant ALVAC virus of claim 4, wherein the DNA from porcine circovirus 2 comprises of the open reading frame 1 (ORF1) of porcine circovirus 2.
8. The recombinant ALVAC virus of claim 4, wherein the DNA from porcine circovirus 2 comprises of the open reading frames 1 and 2 (ORF1 and 2) of porcine circovirus.
9. The recombinant ALVAC virus of claim 4 which is vCP1614 or vCP1615.
10. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 1.
11. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 5.
12. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 6.
13. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 7.
14. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 8.

15. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 9.

16. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 11.

17. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 12.

18. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 13.

19. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 14.

20. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 15.

HindIII (1)
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141 ATAATGACTGCAAATTTGGACGGTTCACATTTTAATCATCACGCGTTCATAAGTTTCAACTGCATAGATC

211 AAAATCTCACTAAAAAGATAGCCGATGTATTTGAGAGAGATTGGACATCTAACTACGCTAAAGAAATTAC

281 AGTTATAAATAATACATAATGGATTTTGTTATCATCAGTTATATTTAACATAAGTACAATAAAAAGTATT

351 AAATAAAAATACTTACTTACGAAAAAATGTCATTATTACAAAAACTATATTTTACAGAACAATCTATAGT
1 Met Ser Leu Leu Gln Lys Leu Tyr Phe Thr Glu Gln Ser Ile Val

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39 Val Val Val Ile Asn Glu Asp Asn Val Leu Leu Ser Thr Arg Leu Leu Ser Phe Asp Lys Ile Leu Phe P

561 TTAACTCCTTTAATAACGGTTTATCAAAATACGAAACTATTAGTGATACAATATTAGATATAGATACTCA
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631 TAATTATTATATACCTAGTTCTTCTTCTTTGTTAGATATTCTAAAAAAAAGAGCGTGTGATTTAGAATTA
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701 GAAGATCTAAATTATGCGTTAATAGGAGACAATAGTAACTTATATTATAAAGATATGACTTACATGAATA
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911 AAAAATGTTATAGAATACTGTTCTCCTGGCTATATATTATGGTTACATGATCTAAAAGCCGCTGCTGAAG
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EcoRI (1223)
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1331 TTTGATGCTAGTTTATTTTTCCAACTCTCTACTAAAAGCAATTATGTAAAATATTGGACAAAGAAAACTT
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1541 ATTCCTGGTTTTGATTTTAAATTCTCCGGAATGATAGATATACTACTGTTTGGAATATTGCATAAGGATA
 389▷ I I ePr oGl yPheAspPheLysPheSer Gl yMet I I eAspI I eLeuLeuPheGl y I I eLeuHi sLysAspA

1611 ATGAGAATATATTTTATCCGAAACGTGTTTCTGTAACTAATATAATATCAGAATCTATCTATGCAGATTT
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1821 TATGCGAAGTAACAGTTTGTAAAGATATAAAAAATCCATTATTATATTCTAAAAAGGATATATCAGCAAA
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 599▷ TyrLysLeuLeuAsnCysHi sMet TyrAsnSer CysLysI I eThr LysCysLysTyrAsnGl nVal I I eT

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 622▷ yrAsnPr o I I e∘ ∘ ∘

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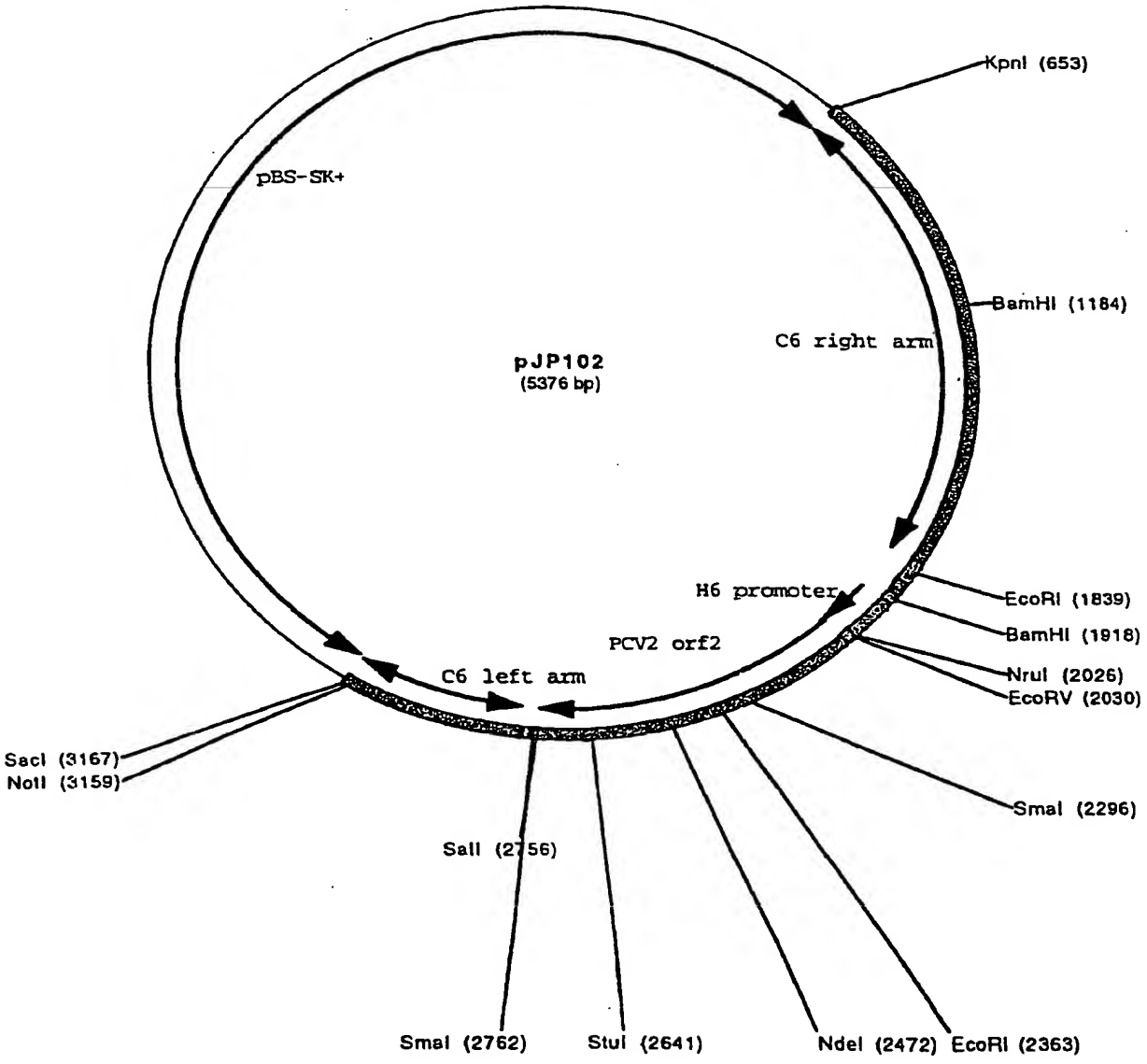
Fig 2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BamHI (1184)
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H6 promoter
EcoRI (1839)
BamHI (1918)
PCV2 orf2
NruI (2026)
EcoRV (2030)
C6 left arm
SacI (3167)
NotI (3159)
SmaI (2296)
SalI (2756)
SmaI (2762)
StuI (2641)
NdeI (2472)
EcoRI (2363)

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351 TAAAAGCTTCCACGTTTTTGTATACATTTCTTTCCATATTAGTAGCTACTACTAAATGATTATCTTCTTT

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701 TAATTATTAATATTAGGTAATTCATTTCCATGTGAAAGATAGACTAATTTTATATCCTTTACCTCCAAAT

771 AATTATTTACATCTCTTAAACAATCTATTTTAATATCATTAACTGGTATTTTATAATATCCAGAAAGGTT

841 TGAAGGGGTTGATGGAATAAGTCTATTAACATCGTTAAGTAAATTATTAATATCATGAATCTTTATTATA

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BamHI (1266)
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SmaI (1644)
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EcoRI (1711)
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NdeI
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SmaI (2110)
SalI (2104)
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2311 CAGTTGAAACTTATGAACGCGTGATGATTAAAATGTGAACCGTCCAAATTTGCAGTCATTATATGAGCGT

2381 ATCTATTATCTACTATCATCATCTTTGAGTTATTAATATCATCTACTTTAGAATTGATAGGAAATATGAA

SacI (2515)
NotI (2507)
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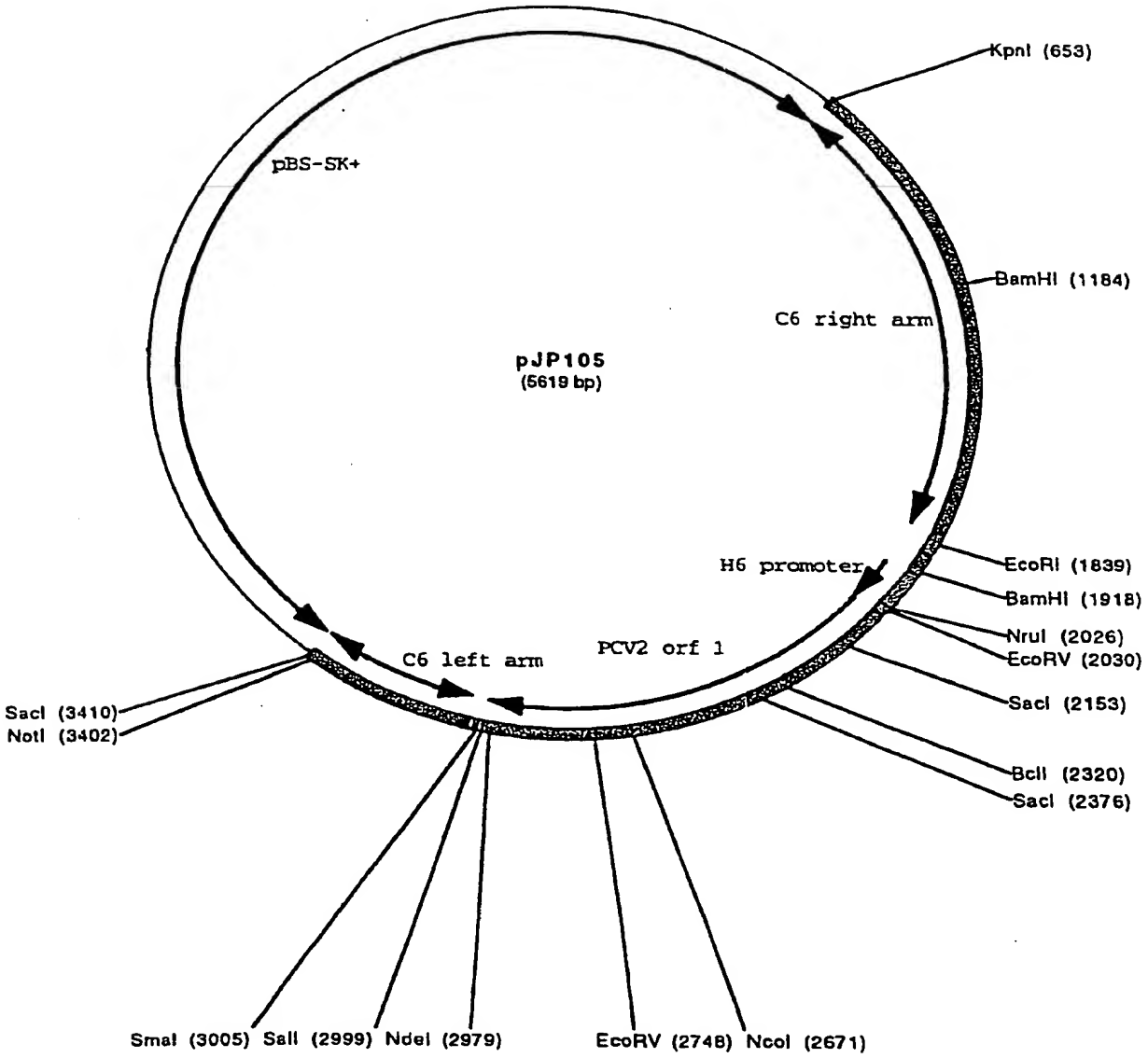
Fig. 3B

Fig. 4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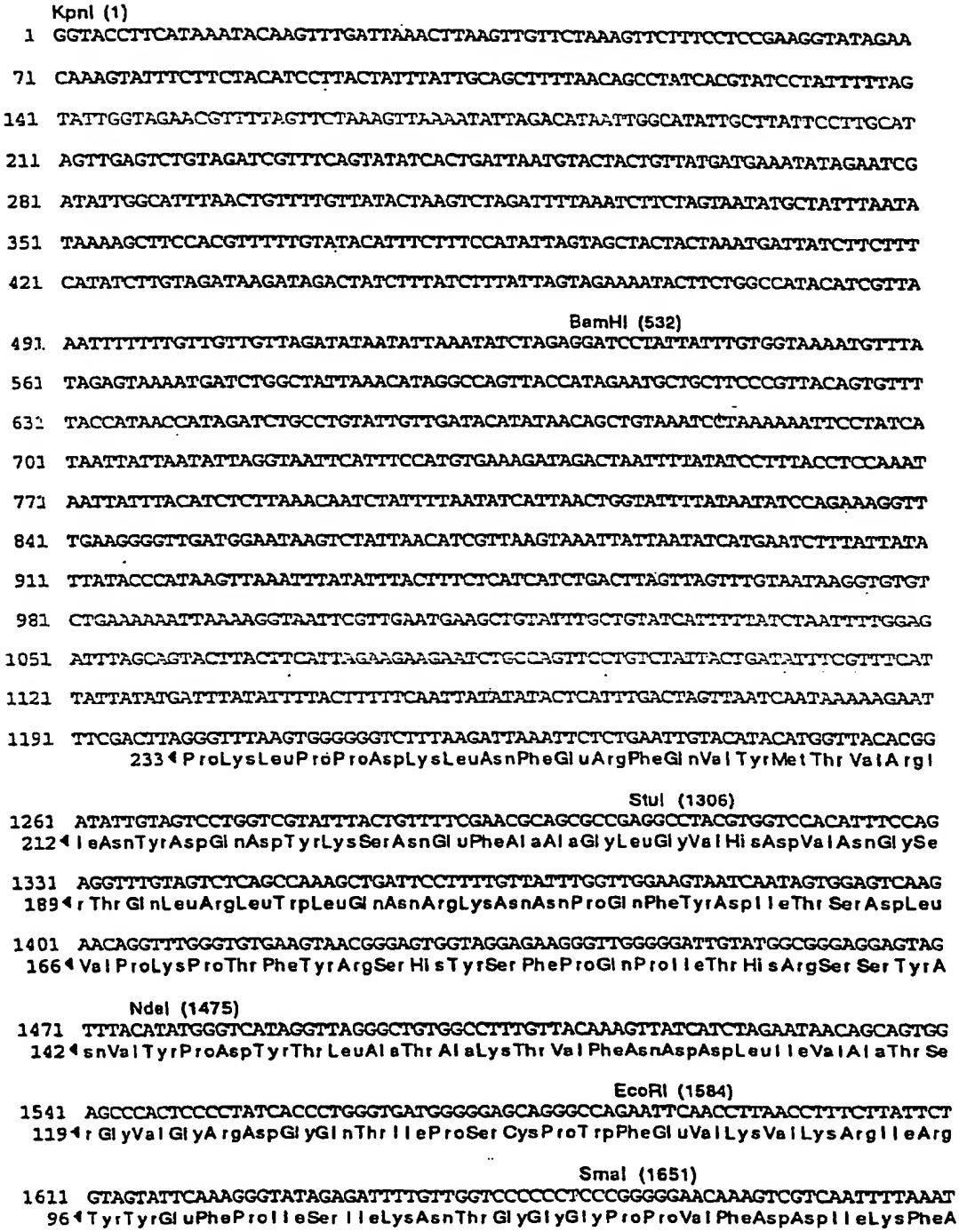
(5619 bp)
KpnI (653)
BamHI (1184)
C6 right arm
H6 promoter
EcoRI (1839)
BamHI (1918)
NruI (2026)
EcoRV (2030)
SacI (2153)
BclI (2320)
SacI (2376)
PCV2 orf 1
C6 left arm
SacI (3410)
NotI (3402)
SmaI (3005)
SalI (2999)
NdeI (2979)
EcoRV (2748)
NcoI (2671)

Fig 6A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BamHI (532)
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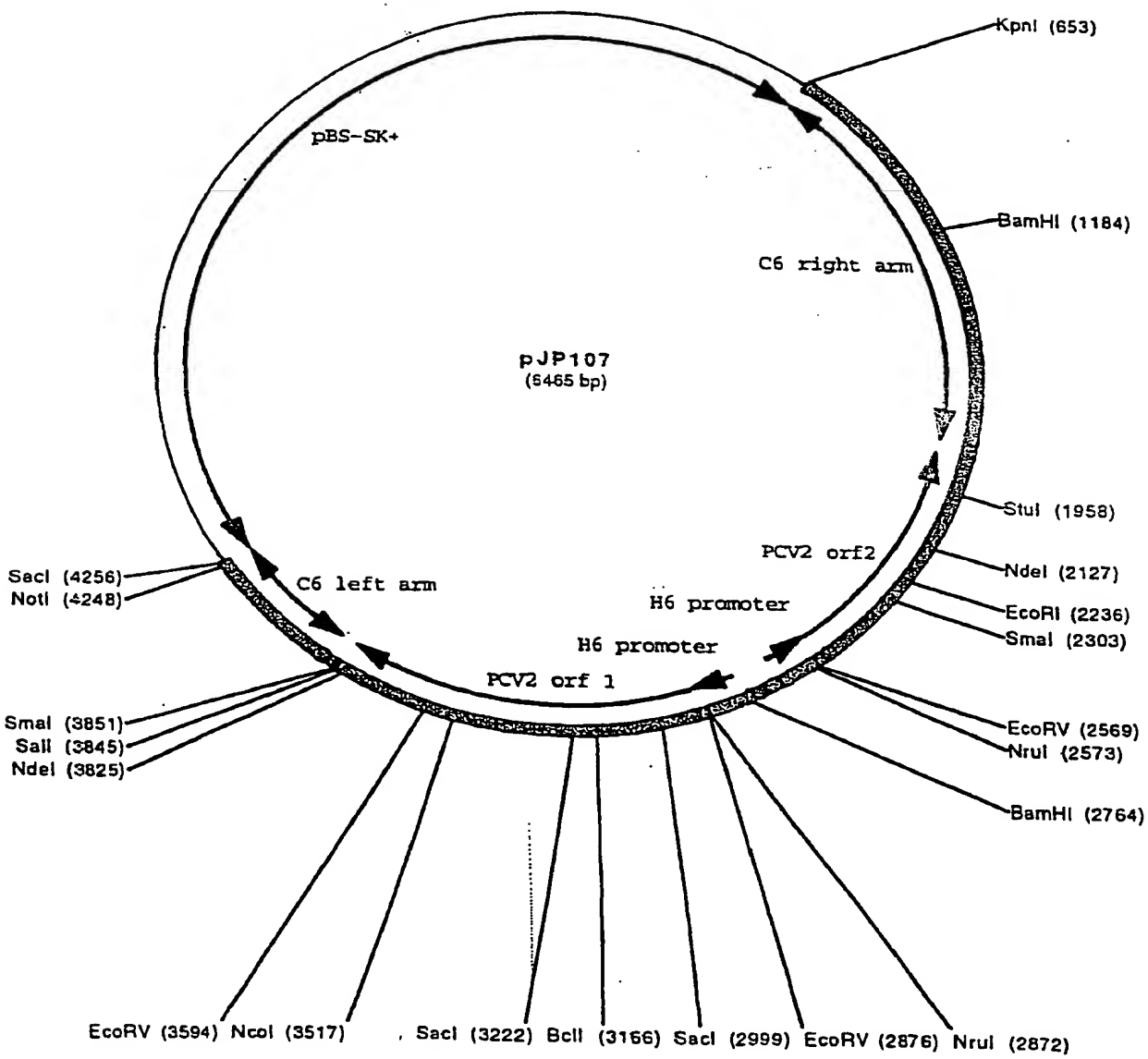
911 TTATACCCATAAGTTAAATTTATATTTACTTTCTCATCATCTGACTTAGTTAGTTTGTAATAAGGTGTGT
981 CTGAAAAAATTAAAAGGTAATTCGTTGAATGAAGCTGTATTTGCTGTATCATTTTTATCTAATTTTGGAG
1051 ATTTAGCAGTACTTACTTCATTAGAAGAAGAATCTGCCAGTTCCTGTCTAATTACTGATATTTCGTTTCAT
1121 TATTATATGATTTATATTTTACTTTTTCAATTATATATACTCATTTGACTAGTTAATCAATAAAAAGAAT
1191 TTCGACTTAGGGTTTAAGTGGGGGGTCTTTAAGATTAAATTCTCTGAATTGTACATACATGGTTACACGG
233 ProLysLeuProProAspLysLeuAsnPheGluArgPheGlnValTyrMetThrValArgI
StuI (1306)
1261 ATATTGTAGTCCTGGTCGTATTTACTGTTTTCGAACGCAGCGCCGAGGCCTACGTGGTCCACATTTCCAG
212 IeAsnTyrAspGlnAspTyrLysSerAsnGluPheAlaAlaGlyLeuGlyValHisAspValAsnGlySe
1331 AGGTTTGTAGTCTCAGCCAAAGCTGATTCCTTTTGTTATTTGGTTGGAAGTAATCAATAGTGGAGTCAAG
189 rThrGlnLeuArgLeuTrpLeuGlnAsnArgLysAsnAsnProGlnPheTyrAspIleThrSerAspLeu
1401 AACAGGTTTGGGTGTGAAGTAACGGGAGTGGTAGGAGAAGGGTTGGGGGATTGTATGGCGGGAGGAGTAG
166 ValProLysProThrPheTyrArgSerHisTyrSerPheProGlnProIleThrHisArgSerSerTyrA
NdeI (1475)
1471 TTTACATATGGGTCATAGGTTAGGGCTGTGGCCTTTGTTACAAAGTTATCATCTAGAATAACAGCAGTGG
142 snValTyrProAspTyrThrLeuAlaThrAlaLysThrValPheAsnAspAspLeuIleValAlaThrSe
EcoRI (1584)
1541 AGCCCACTCCCCTATCACCCTGGGTGATGGGGGAGCAGGGCCAGAATTCAACCTTAACCTTTCTTATTCT
119 rGlyValGlyArgAspGlyGlnThrIleProSerCysProTrpPheGluValLysValLysArgIleArg
SmaI (1651)
1611 GTAGTATTCAAAGGGTATAGAGATTTTGTTGGTCCCCCCTCCCGGGGGAACAAAGTCGTCAATTTTAAAT
96 TyrTyrGluPheProIleSerIleLysAsnThrGlyGlyGlyProProValPheAspAspIleLysPheA

Fig 5


pBS-SK+
pJP107
(5465 bp)
KpnI (653)
BamHI (1184)
C6 right arm
StuI (1958)
PCV2 orf2
NdeI (2127)
EcoRI (2236)
SmaI (2303)
H6 promoter
H6 promoter
PCV2 orf 1
EcoRV (2569)
NruI (2573)
BamHI (2764)
C6 left arm
SacI (4256)
NotI (4248)
SmaI (3851)
SalI (3845)
NdeI (3825)
EcoRV (3594)
NcoI (3517)
SacI (3222)
BclI (3166)
SacI (2999)
EcoRV (2876)
NruI (2872)

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168[ CTCATCATGTCCACCGCCCAGGAGGGCGTTGTGACTGTGGTACGCTTGACAGTATATCCGAAGGTGCGGG
 72[ rgMetMetAspValAlaTrpSerProThrThrValThrThrArgLysValThrTyrGlyPheThrArgSe

1751 AGAGGCGGGTGTTGAAGATGCCATTTTTCCTTCTCCAACGGTAGCGGTGGCGGGGGTGGACGAGCCAGGG
 49[ rLeuArgThrAsnPheIleGlyAsnLysArgArgTrpArgTyrArgHisArgProHisValLeuTrpPro

1821 GCGGCGGCGGAGGATCTGGCCAAGATGGCTGCGGGGGCGGTGTCTTCTTCTGCGGTAACGCCTCCTTGGA
 26[ArgArgArgLeuIleGlnGlyLeuHisSerArgProArgHisArgArgArgArgTyrArgArgArgProT

                                        NruI (1921)
                                  EcoRV (1917)
1891 TACGTCATTACGATACAAACTTAACGGATATCGCGATAATGAAATAATTTATGATTATTTCTCGCTTTCA
  2[yrThrMet

1961 ATTTAACACAACCCTCAAGAACCTTTGTATTTATTTTCACTTTTTAAGTATAGAATAAAGAAGCTGGGGG

2031 ATCAATTCCTGCAGCCCTGCAGCTAATTAATTAAGCTACAAATAGTTTCGTTTTCACCTTGTCTAATAAC

                BamHI (2112)
2101 TAATTAATTAAGGATCCCCCAGCTTCTTTATTCTATACTTAAAAAGTGAAAATAAATACAAAGGTTCTTG

                                                          EcoRV (2224)
                                                    NruI (2220)
2171 AGGGTTGTGTTAAATTGAAAGCGAGAAATAATCATAAATTATTTCATTATCGCGATATCCGTTAAGTTTG

2241 TATCGTAATGCCCAGCAAGAAGAATGGAAGAAGCGGACCCCAACCACATAAAAGGTGGGTGTTCACGCTG
     1[MetProSerLysLysAsnGlyArgSerGlyProGlnProHisLysArgTrpValPheThrLeu

                                        SacI (2347)
2311 AATAATCCTTCCGAAGACGAGCGCAAGAAAATACGGGAGCTCCCAATCTCCCTATTTGATTATTTTATTG
 22[AsnAsnProSerGluAspGluArgLysLysIleArgGluLeuProIleSerLeuPheAspTyrPheIleV

2381 TTGGCGAGGAGGGTAATGAGGAAGGACGAACACCTCACCTCCAGGGGTTCGCTAATTTTGTGAAGAAGCA
 45[alGlyGluGluGlyAsnGluGluGlyArgThrProHisLeuGlnGlyPheAlaAsnPheValLysLysGl

                                                                    BclI (2514)
2451 AACTTTTAATAAAGTGAAGTGGTATTTGGGTGCCCGCTGCCACATCGAGAAAGCCAAAGGAACTGATCAG
 68[nThrPheAsnLysValLysTrpTyrLeuGlyAlaArgCysHisIleGluLysAlaLysGlyThrAspGln

                                                    SacI (2570)
2521 CAGAATAAAGAATATTGCAGTAAAGAAGGCAACTTACTTATTGAATGTGGAGCTCCTCGATCTCAAGGAC
 92[GlnAsnLysGluTyrCysSerLysGluGlyAsnLeuLeuIleGluCysGlyAlaProArgSerGlnGlyG

2591 AACGGAGTGACCTGTCTACTGCTGTGAGTACCTTGTTGGAGAGCGGGAGTCTGGTGACCGTTGCAGAGCA
115[InArgSerAspLeuSerThrAlaValSerThrLeuLeuGluSerGlySerLeuValThrValAlaGluGl

2661 GCACCCTGTAACGTTTGTCAGAAATTTCCGCGGGCTGGCTGAACTTTTGAAAGTGAGCGGGAAAATGCAG
138[nHisProValThrPheValArgAsnPheArgGlyLeuAlaGluLeuLeuLysValSerGlyLysMetGln

2731 AAGCGTGATTGGAAGACCAATGTACACGTCATTGTGGGGCCACCTGGGTGTGGTAAAAGCAAATGGGCTG
162[LysArgAspTrpLysThrAsnValHisValIleValGlyProProGlyCysGlyLysSerLysTrpAlaA

                                                                    NcoI (2865
2801 CTAATTTTGCAGACCCGGAAACCACATACTGGAAACCACCTAGAAACAAGTGGTGGGATGGTTACCATGG
185[IaAsnPheAlaAspProGluThrThrTyrTrpLysProProArgAsnLysTrpTrpAspGlyTyrHisGl

2871 TGAAGAAGTGGTTGTTATTGATGACTTTTATGGCTGGCTGCCGTGGGATGATCTACTGAGACTGTGTGAT
208[yGluGluValValValIleAspAspPheTyrGlyTrpLeuProTrpAspAspLeuLeuArgLeuCysAsp

      EcoRV (2942)
2941 CGATATCCATTGACTGTAGAGACTAAAGGTGGAACTGTACCTTTTTTGGCCCGCAGTATTCTGATTACCA
232[ArgTyrProLeuThrValGluThrLysGlyGlyThrValProPheLeuAlaArgSerIleLeuIleThrS
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3011  GCAATCAGACCCCGTTGGAATGGTACTCCTCAACTGCTGTCCCAGCTGTAGAAGCTCTCTATCGGAGGAT
255▷  erAsnGlnThrProLeuGluTrpTyrSerSerThrAlaValProAlaValGluAlaLeuTyrArgArgIl

3081  TACTTCCTTGGTATTTTGGAAGAATGCTACAGAACAATCCACGGAGGAAGGGGGCCAGTTCGTCACCCTT
278▷  eThrSerLeuValPheTrpLysAsnAlaThrGluGlnSerThrGluGluGlyGlyGlnPheValThrLeu

                                                                 SmaI (3199)
                                    NdeI (3173)            SalI (3193)
3151  TCCCCCCCATGCCCTGAATTTCCATATGAAATAAATTACTGAGTCGACCCCGGGTTTTTATAGCTAATTA
302▷  SerProProCysProGluPheProTyrGluIleAsnTyr

3221  GTCATTTTTTCGTAAGTAAGTATTTTTATTTAATACTTTTTATTGTACTTATGTTAAATATAACTGATGA

3291  TAACAAAATCCATTATGTATTATTTATAACTGTAATTTCTTTAGCGTAGTTAGATGTCCAATCTCTCTCA

3361  AATACATCGGCTATCTTTTTAGTGAGATTTTGATCTATGCAGTTGAAACTTATGAACGCGTGATGATTAA

3431  AATGTGAACCGTCCAAATTTGCAGTCATTATATGAGCGTATCTATTATCTACTATCATCATCTTTGAGTT

3501  ATTAATATCATCTACTTTAGAATTGATAGGAAATATGAATACCTTTGTAGTAATATCTATACTATCTACA

                                              SacI (3604)
                                      NotI (3596)
3571  CCTAACTCATTAAGACTTTTGATAGGCGGCCGCGAGCTC
```

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Bublot, Michel
Perez, Jennifer M.
Charreyre, Catherine E.

(ii) TITLE OF INVENTION: Porcine Circovirus 2 Recombinant Poxvirus

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ~~Virogenetics Inc.~~ Frommer Lawrence & Haug LLP
(B) STREET: ~~465 Jordan Road~~ 745 Fifth Avenue
(C) CITY: ~~Troy~~ NY
(D) STATE: ~~NY~~ NY
(E) COUNTRY: USA
(F) ZIP: ~~12180~~ 10151

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: ~~Howe, Timothy R.~~ Kowalski, Thomas J.
(B) REGISTRATION NUMBER: ~~39,228~~ 32,147
(C) REFERENCE/DOCKET NUMBER: ~~TH015~~ 454313-2511.1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: ~~(570) 586-1022~~ 212-588-0800
(B) TELEFAX: ~~(570) 895-2702~~ 212-588-0500

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3701 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGCTTCTAT CAAAAGTCTT AATGAGTTAG GTGTAGATAG TATAGATATT ACTACAAAGG
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TATTCATATT TCCTATCAAT TCTAAAGTAG ATGATATTAA TAACTCAAAG ATGATGATAG
120

TAGATAATAG ATACGCTCAT ATAATGACTG CAAATTTGGA CGGTTCACAT TTTAATCATC
180

ACGCGTTCAT AAGTTTCAAC TGCATAGATC AAAATCTCAC TAAAAAGATA GCCGATGTAT
240

TTGAGAGAGA TTGGACATCT AACTACGCTA AAGAAATTAC AGTTATAAAT AATACATAAT
300

GGATTTTGTT ATCATCAGTT ATATTTAACA TAAGTACAAT AAAAAGTATT AAATAAAAAT
360

ACTTACTTAC GAAAAAATGT CATTATTACA AAAACTATAT TTTACAGAAC AATCTATAGT
420

AGAGTCCTTT AAGAGTTATA ATTTAAAAGA TAACCATAAT GTAATATTTA CCACATCAGA
480

TGATGATACT GTTGTAGTAA TAAATGAAGA TAATGTACTG TTATCTACAA GATTATTATC
540

ATTTGATAAA ATTCTGTTTT TTAACTCCTT TAATAACGGT TTATCAAAAT ACGAAACTAT
600

TAGTGATACA ATATTAGATA TAGATACTCA TAATTATTAT ATACCTAGTT CTTCTTCTTT

660

GTTAGATATT CTAAAAAAAA GAGCGTGTGA TTTAGAATTA GAAGATCTAA ATTATGCGTT
720

AATAGGAGAC AATAGTAACT TATATTATAA AGATATGACT TACATGAATA ATTGGTTATT
780

TACTAAAGGA TTATTAGATT ACAAGTTTGT ATTATTGCGC GATGTAGATA AATGTTACAA
840

ACAGTATAAT AAAAAGAATA CTATAATAGA TATAATACAT CGCGATAACA GACAGTATAA
900

CATATGGGTT AAAAATGTTA TAGAATACTG TTCTCCTGGC TATATATTAT GGTTACATGA
960

TCTAAAAGCC GCTGCTGAAG ATGATTGGTT AAGATACGAT AACCGTATAA ACGAATTATC
1020

TGCGGATAAA TTATACACTT TCGAGTTCAT AGTTATATTA GAAAATAATA TAAAACATTT
1080

ACGAGTAGGT ACAATAATTG TACATCCAAA CAAGATAATA GCTAATGGTA CATCTAATAA
1140

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1200

AATATTGGCC GGATATTTTT TAGAATTCTT TGAGACCACT ATTTTATCAG AATTTATTTC
1260

TTCATCTTCT GAATGGGTAA TGAATAGTAA CTGTTTAGTA CACCTGAAAA CAGGGTATGA
1320

AGCTATACTC TTTGATGCTA GTTTATTTTT CCAACTCTCT ACTAAAAGCA ATTATGTAAA
1380

ATATTGGACA AAGAAAACTT TGCAGTATAA GAACTTTTTT AAAGACGGTA AACAGTTAGC
1440

AAAATATATA ATTAAGAAAG ATAGTCAGGT GATAGATAGA GTATGTTATT TACACGCAGC
1500

TGTATATAAT CACGTAACTT ACTTAATGGA TACGTTTAAA ATTCCTGGTT TTGATTTTAA
1560

ATTCTCCGGA ATGATAGATA TACTACTGTT TGGAATATTG CATAAGGATA ATGAGAATAT
1620

ATTTTATCCG AAACGTGTTT CTGTAACTAA TATAATATCA GAATCTATCT ATGCAGATTT
1680

TTACTTTATA TCAGATGTTA ATAAATTCAG TAAAAAGATA GAATATAAAA CTATGTTTCC
1740

TATACTCGCA GAAAACTACT ATCCAAAAGG AAGGCCCTAT TTTACACATA CATCTAACGA
1800

AGATCTTCTG TCTATCTGTT TATGCGAAGT AACAGTTTGT AAAGATATAA AAAATCCATT
1860

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1920

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1980

GCCTGAAAAG ATAAAAATAT TTAATTCTAA TCCTACATAC ATTAGATTAT TACTAACAGA
2040

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2100

TACCAGAGAT GGAGTCAGAA ATAATTTACC TATAATTTCT TTTATCGTCA GTTATTGTAG
2160

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2220

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2280

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2340

TTCTAATGAA GTAAGTACTG CTAAATCTCC AAAATTAGAT AAAAATGATA CAGCAAATAC
2400

AGCTTCATTC AACGAATTAC CTTTTAATTT TTTCAGACAC ACCTTATTAC AAACTAACTA
2460

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2520

ATGATATTAA TAATTTACTT AACGATGTTA ATAGACTTAT TCCATCAACC CCTTCAAACC
2580

TTTCTGGATA TTATAAAATA CCAGTTAATG ATATTAAAAT AGATTGTTTA AGAGATGTAA
2640

ATAATTATTT GGAGGTAAAG GATATAAAAT TAGTCTATCT TTCACATGGA AATGAATTAC
2700

CTAATATTAA TAATTATGAT AGGAATTTTT TAGGATTTAC AGCTGTTATA TGTATCAACA
2760

ATACAGGCAG ATCTATGGTT ATGGTAAAAC ACTGTAACGG GAAGCAGCAT TCTATGGTAA
2820

CTGGCCTATG TTTAATAGCC AGATCATTTT ACTCTATAAA CATTTTACCA CAAATAATAG
2880

GATCCTCTAG ATATTTAATA TTATATCTAA CAACAACAAA AAAATTTAAC GATGTATGGC
2940

CAGAAGTATT TTCTACTAAT AAAGATAAAG ATAGTCTATC TTATCTACAA GATATGAAAG
3000

AAGATAATCA TTTAGTAGTA GCTACTAATA TGGAAAGAAA TGTATACAAA AACGTGGAAG
3060

CTTTTATATT AAATAGCATA TTACTAGAAG ATTTAAAATC TAGACTTAGT ATAACAAAAC
3120

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3180

TACTGAAACG ATCTACAGAC TCAACTATGC AAGGAATAAG CAATATGCCA ATTATGTCTA

3240

ATATTTTAAC TTTAGAACTA AAACGTTCTA CCAATACTAA AAATAGGATA CGTGATAGGC
3300

TGTTAAAAGC TGCAATAAAT AGTAAGGATG TAGAAGAAAT ACTTTGTTCT ATACCTTCGG
3360

AGGAAAGAAC TTTAGAACAA CTTAAGTTTA ATCAAACTTG TATTTATGAA CACTATAAAA
3420

AAATTATGGA AGATACAAGT AAAAGAATGG ATGTTGAATG TCGTAGTTTA GAACATAACT
3480

ATACGGCTAA CTTATATAAA GTGTACGGAC AAAACGAATA TATGATTACT TATATACTAG
3540

CTCTCATAAG TAGGATTAAT AATATTATAG AAACTTTAAA ATATAATCTG GTGGGGCTAG
3600

ACGAATCTAC AATACGTAAT ATAAATTATA TAATTTCACA AAGAACAAAA AAAAATCAAG
3660

TTTCTAATAC CTTATAGATA AACTATATTT TTTACCACTG A
3701

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATCATCGAGC TCGCGGCCGC CTATCAAAAG TCTTAATGAG TT
42

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCCTCG AGCTGCAGCC CGGGTTTTTA TAGCTAATTA GTCATTTTTT CGTAAGTAAG
60

TATTTTTATT TAA
73

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCCGGGCTGC AGCTCGAGGA ATTCTTTTTA TTGATTAACT AGTCAAATGA GTATATATAA
60

TTGAAAAAGT AA
72

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATGATGGTA CCTTCATAAA TACAAGTTTG ATTAAACTTA AGTTG
45

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATCATCATG ATATCCGTTA AGTTTGTATC GTAATGACGT ATCCAAGGAG GCG
53

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TACTACTACG TCGACTTAGG GTTTAAGTGG GGGGTC
36

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2520 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGTACCTTCA TAAATACAAG TTTGATTAAA CTTAAGTTGT TCTAAAGTTC TTTCCTCCGA
60

AGGTATAGAA CAAAGTATTT CTTCTACATC CTTACTATTT ATTGCAGCTT TTAACAGCCT
120

ATCACGTATC CTATTTTTAG TATTGGTAGA ACGTTTTAGT TCTAAAGTTA AAATATTAGA
180

CATAATTGGC ATATTGCTTA TTCCTTGCAT AGTTGAGTCT GTAGATCGTT TCAGTATATC
240

ACTGATTAAT GTACTACTGT TATGATGAAA TATAGAATCG ATATTGGCAT TTAACTGTTT
300

TGTTATACTA AGTCTAGATT TTAAATCTTC TAGTAATATG CTATTTAATA TAAAAGCTTC
360

CACGTTTTTG TATACATTTC TTTCCATATT AGTAGCTACT ACTAAATGAT TATCTTCTTT
420

CATATCTTGT AGATAAGATA GACTATCTTT ATCTTTATTA GTAGAAAATA CTTCTGGCCA
480

TACATCGTTA AATTTTTTTG TTGTTGTTAG ATATAATATT AAATATCTAG AGGATCCTAT
540

TATTTGTGGT AAAATGTTTA TAGAGTAAAA TGATCTGGCT ATTAAACATA GGCCAGTTAC
600

CATAGAATGC TGCTTCCCGT TACAGTGTTT TACCATAACC ATAGATCTGC CTGTATTGTT
660

GATACATATA ACAGCTGTAA ATCCTAAAAA ATTCCTATCA TAATTATTAA TATTAGGTAA
720

TTCATTTCCA TGTGAAAGAT AGACTAATTT TATATCCTTT ACCTCCAAAT AATTATTTAC
780

ATCTCTTAAA CAATCTATTT TAATATCATT AACTGGTATT TTATAATATC CAGAAAGGTT
840

TGAAGGGGTT GATGGAATAA GTCTATTAAC ATCGTTAAGT AAATTATTAA TATCATGAAT
900

CTTTATTATA TTATACCCAT AAGTTAAATT TATATTTACT TTCTCATCAT CTGACTTAGT
960

TAGTTTGTAA TAAGGTGTGT CTGAAAAAAT TAAAAGGTAA TTCGTTGAAT GAAGCTGTAT
1020

TTGCTGTATC ATTTTTATCT AATTTTGGAG ATTTAGCAGT ACTTACTTCA TTAGAAGAAG
1080

AATCTGCCAG TTCCTGTCTA TTACTGATAT TTCGTTTCAT TATTATATGA TTTATATTTT
1140

ACTTTTTCAA TTATATATAC TCATTTGACT AGTTAATCAA TAAAAAGAAT TCCTGCAGCC
1200

CTGCAGCTAA TTAATTAAGC TACAAATAGT TTCGTTTTCA CCTTGTCTAA TAACTAATTA
1260

ATTAAGGATC CCCCAGCTTC TTTATTCTAT ACTTAAAAAG TGAAAATAAA TACAAAGGTT
1320

CTTGAGGGTT GTGTTAAATT GAAAGCGAGA AATAATCATA AATTATTTCA TTATCGCGAT
1380

ATCCGTTAAG TTTGTATCGT AATGACGTAT CCAAGGAGGC GTTACCGCAG AAGAAGACAC
1440

CGCCCCCGCA GCCATCTTGG CCAGATCCTC CGCCGCCGCC CCTGGCTCGT CCACCCCCGC
1500

CACCGCTACC GTTGGAGAAG GAAAAATGGC ATCTTCAACA CCCGCCTCTC CCGCACCTTC
1560

GGATATACTG TCAAGCGTAC CACAGTCACA ACGCCCTCCT GGGCGGTGGA CATGATGAGA
1620

TTTAAAATTG ACGACTTTGT TCCCCCGGGA GGGGGGACCA ACAAAATCTC TATACCCTTT
1680

GAATACTACA GAATAAGAAA GGTTAAGGTT GAATTCTGGC CCTGCTCCCC CATCACCCAG
1740

GGTGATAGGG GAGTGGGCTC CACTGCTGTT ATTCTAGATG ATAACTTTGT AACAAAGGCC
1800

ACAGCCCTAA CCTATGACCC ATATGTAAAC TACTCCTCCC GCCATACAAT CCCCCAACCC
1860

TTCTCCTACC ACTCCCGTTA CTTCACACCC AAACCTGTTC TTGACTCCAC TATTGATTAC
1920

TTCCAACCAA ATAACAAAAG GAATCAGCTT TGGCTGAGAC TACAAACCTC TGGAAATGTG
1980

GACCACGTAG GCCTCGGCGC TGCGTTCGAA AACAGTAAAT ACGACCAGGA CTACAATATC
2040

CGTGTAACCA TGTATGTACA ATTCAGAGAA TTTAATCTTA AAGACCCCCC ACTTAAACCC
2100

TAAGTCGACC CCGGGTTTTT ATAGCTAATT AGTCATTTTT TCGTAAGTAA GTATTTTTAT
2160

TTAATACTTT TTATTGTACT TATGTTAAAT ATAACTGATG ATAACAAAAT CCATTATGTA
2220

TTATTTATAA CTGTAATTTC TTTAGCGTAG TTAGATGTCC AATCTCTCTC AAATACATCG
2280

GCTATCTTTT TAGTGAGATT TTGATCTATG CAGTTGAAAC TTATGAACGC GTGATGATTA
2340

AAATGTGAAC CGTCCAAATT TGCAGTCATT ATATGAGCGT ATCTATTATC TACTATCATC
2400

ATCTTTGAGT TATTAATATC ATCTACTTTA GAATTGATAG GAAATATGAA TACCTTTGTA
2460

GTAATATCTA TACTATCTAC ACCTAACTCA TTAAGACTTT TGATAGGCGG CCGCGAGCTC

2520

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CATCATCATT CGCGATATCC GTTAAGTTTG TATCGTAATG CCCAGCAAGA AGAATGG
57

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TACTACTACG TCGACTCAGT AATTTATTTC ATATGG
36

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3609 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGTACCTTCA TAAATACAAG TTTGATTAAA CTTAAGTTGT TCTAAAGTTC TTTCCTCCGA
60

AGGTATAGAA CAAAGTATTT CTTCTACATC CTTACTATTT ATTGCAGCTT TTAACAGCCT
120

ATCACGTATC CTATTTTTAG TATTGGTAGA ACGTTTTAGT TCTAAAGTTA AAATATTAGA
180

CATAATTGGC ATATTGCTTA TTCCTTGCAT AGTTGAGTCT GTAGATCGTT TCAGTATATC
240

ACTGATTAAT GTACTACTGT TATGATGAAA TATAGAATCG ATATTGGCAT TTAACTGTTT
300

TGTTATACTA AGTCTAGATT TTAAATCTTC TAGTAATATG CTATTTAATA TAAAAGCTTC
360

CACGTTTTTG TATACATTTC TTTCCATATT AGTAGCTACT ACTAAATGAT TATCTTCTTT
420

CATATCTTGT AGATAAGATA GACTATCTTT ATCTTTATTA GTAGAAAATA CTTCTGGCCA
480

TACATCGTTA AATTTTTTTG TTGTTGTTAG ATATAATATT AAATATCTAG AGGATCCTAT
540

TATTTGTGGT AAAATGTTTA TAGAGTAAAA TGATCTGGCT ATTAAACATA GGCCAGTTAC
600

CATAGAATGC TGCTTCCCGT TACAGTGTTT TACCATAACC ATAGATCTGC CTGTATTGTT
660

GATACATATA ACAGCTGTAA ATCCTAAAAA ATTCCTATCA TAATTATTAA TATTAGGTAA
720

TTCATTTCCA TGTGAAAGAT AGACTAATTT TATATCCTTT ACCTCCAAAT AATTATTTAC
780

ATCTCTTAAA CAATCTATTT TAATATCATT AACTGGTATT TTATAATATC CAGAAAGGTT
840

TGAAGGGGTT GATGGAATAA GTCTATTAAC ATCGTTAAGT AAATTATTAA TATCATGAAT
900

CTTTATTATA TTATACCCAT AAGTTAAATT TATATTTACT TTCTCATCAT CTGACTTAGT
960

TAGTTTGTAA TAAGGTGTGT CTGAAAAAAT TAAAAGGTAA TTCGTTGAAT GAAGCTGTAT
1020

TTGCTGTATC ATTTTTATCT AATTTTGGAG ATTTAGCAGT ACTTACTTCA TTAGAAGAAG
1080

AATCTGCCAG TTCCTGTCTA TTACTGATAT TTCGTTTCAT TATTATATGA TTTATATTTT
1140

ACTTTTTCAA TTATATATAC TCATTTGACT AGTTAATCAA TAAAAAGAAT TTCGACTTAG
1200

GGTTTAAGTG GGGGGTCTTT AAGATTAAAT TCTCTGAATT GTACATACAT GGTTACACGG
1260

ATATTGTAGT CCTGGTCGTA TTTACTGTTT TCGAACGCAG CGCCGAGGCC TACGTGGTCC
1320

ACATTTCCAG AGGTTTGTAG TCTCAGCCAA AGCTGATTCC TTTTGTTATT TGGTTGGAAG
1380

TAATCAATAG TGGAGTCAAG AACAGGTTTG GGTGTGAAGT AACGGGAGTG GTAGGAGAAG
1440

GGTTGGGGGA TTGTATGGCG GGAGGAGTAG TTTACATATG GGTCATAGGT TAGGGCTGTG
1500

GCCTTTGTTA CAAAGTTATC ATCTAGAATA ACAGCAGTGG AGCCCACTCC CCTATCACCC
1560

TGGGTGATGG GGGAGCAGGG CCAGAATTCA ACCTTAACCT TTCTTATTCT GTAGTATTCA
1620

AAGGGTATAG AGATTTTGTT GGTCCCCCCT CCCGGGGGAA CAAAGTCGTC AATTTTAAAT
1680

CTCATCATGT CCACCGCCCA GGAGGGCGTT GTGACTGTGG TACGCTTGAC AGTATATCCG
1740

AAGGTGCGGG AGAGGCGGGT GTTGAAGATG CCATTTTTCC TTCTCCAACG GTAGCGGTGG
1800

CGGGGGTGGA CGAGCCAGGG GCGGCGGCGG AGGATCTGGC CAAGATGGCT GCGGGGGCGG
1860

TGTCTTCTTC TGCGGTAACG CCTCCTTGGA TACGTCATTA CGATACAAAC TTAACGGATA
1920

TCGCGATAAT GAAATAATTT ATGATTATTT CTCGCTTTCA ATTTAACACA ACCCTCAAGA
1980

ACCTTTGTAT TTATTTTCAC TTTTTAAGTA TAGAATAAAG AAGCTGGGGG ATCAATTCCT
2040

GCAGCCCTGC AGCTAATTAA TTAAGCTACA AATAGTTTCG TTTTCACCTT GTCTAATAAC
2100

TAATTAATTA AGGATCCCCC AGCTTCTTTA TTCTATACTT AAAAAGTGAA AATAAATACA
2160

AAGGTTCTTG AGGGTTGTGT TAAATTGAAA GCGAGAAATA ATCATAAATT ATTTCATTAT
2220

CGCGATATCC GTTAAGTTTG TATCGTAATG CCCAGCAAGA AGAATGGAAG AAGCGGACCC
2280

CAACCACATA AAAGGTGGGT GTTCACGCTG AATAATCCTT CCGAAGACGA GCGCAAGAAA
2340

ATACGGGAGC TCCCAATCTC CCTATTTGAT TATTTTATTG TTGGCGAGGA GGGTAATGAG
2400

GAAGGACGAA CACCTCACCT CCAGGGGTTC GCTAATTTTG TGAAGAAGCA AACTTTTAAT
2460

AAAGTGAAGT GGTATTTGGG TGCCCGCTGC CACATCGAGA AAGCCAAAGG AACTGATCAG
2520

CAGAATAAAG AATATTGCAG TAAAGAAGGC AACTTACTTA TTGAATGTGG AGCTCCTCGA

PAGE MISSING AT TIME OF PUBLICATION

TGATGATTAA AATGTGAACC GTCCAAATTT GCAGTCATTA TATGAGCGTA TCTATTATCT
3480

ACTATCATCA TCTTTGAGTT ATTAATATCA TCTACTTTAG AATTGATAGG AAATATGAAT
3540

ACCTTTGTAG TAATATCTAT ACTATCTACA CCTAACTCAT TAAGACTTTT GATAGGCGGC
3600

CGCGAGCTC
3609

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CATCATCATG ATATCCGTTA AGTTTGTATC GTAATGACGT GGCCAAGGAG GCG
53

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TACTACTACG TCGACTTATT TATTTAGAGG GTCTTTTAGG
40

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW). Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM). European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

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9 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/77216 A3

(54) Title: PORCINE CIRCOVIRUS VACCINE IN RECOMBINANT POXVIRUS

(57) Abstract: What is described is a recombinant poxvirus, such as avipox virus, containing foreign DNA from porcine circovirus 2. What are also described are immunological compositions containing the recombinant poxvirus for inducing an immunological response in a host animal to which the immunological composition is administered. Also described are methods of treating or preventing disease caused by porcine circovirus 2 by administering the immunological compositions of the invention to an animal in need of treatment or susceptible to infection by porcine circovirus 2.

INTERNATIONAL SEARCH REPORT

Int[illegible]onal Application No
PCT/IB 00/00882

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/34 C07K14/01 C12N7/01 C12N15/863 A61K39/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBL, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	FR 2 769 322 A (MERIAL SAS) 9 April 1999 (1999-04-09) page 6, line 30 -page 7, line 6	1-3,10 4-9, 11-20
Y	FRIES L. F. ET AL.: "Human safety and immunogenicity of a canarypox-rabies glycoprotein recombinant vaccine: an alternative poxvirus vector system" VACCINE, vol. 14, no. 5, 1 April 1996 (1996-04-01), pages 428-434, XP004057299 ISSN: 0264-410X page 428, right-hand column -page 429, left-hand column, paragraph 3 -/—	4-9, 11-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2001	13/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

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INTERNATIONAL SEARCH REPORT

Int[illegible]onal Application No
PCT/IB 00/00882

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MEEHAN B. M. ET AL.: "Characterization of novel circovirus DNAs associated with wasting syndromes in pigs." JOURNAL OF GENERAL VIROLOGY, vol. 79, no. 9, 1998, pages 2171-2179, XP002160906 ISSN: 0022-1317 cited in the application figure 5	1-20
A	US 5 756 103 A (DE TAISNE CHARLES ET AL) 26 May 1998 (1998-05-26) column 5, line 65 -column 6, line 49 example 67	1-20
A	TAYLOR J. ET AL.: "NONREPLICATING VIRAL VECTORS AS POTENTIAL VACCINES: RECOMBINANT CANARYPOX VIRUS EXPRESSING MEASLES VIRUS FUSION (F) AND HEMAGGLUTININ (HA) GLYCOPROTEINS" VIROLOGY, vol. 187, no. 1, 1 March 1992 (1992-03-01), pages 321-328, XP002053145 ISSN: 0042-6822 cited in the application page 312, left-hand column, line 20 - line 24	3-9, 11-20
A	TAYLOR J. ET AL.: "Biological and immunogenic properties of a canarypox-rabies recombinant, ALVAC-RG (vCP65) in non-avian species." VACCINE, vol. 13, no. 6, 1995, pages 539-549, XP002160907 ISSN: 0264-410X the whole document	3-9, 11-20
A	TAYLOR J. ET AL.: "RECOMBINANT FOWLPOX VIRUS INDUCING PROTECTIVE IMMUNITY IN NON-AVIAN SPECIES" VACCINE, vol. 6, no. 6, 1988, pages 497-503, XP002160908 ISSN: 0264-410X cited in the application the whole document	3-9, 11-20

INTERNATIONAL SEARCH REPORT

information on patent family members

Int. onal Application No
PCT/IB 00/00882

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2769322 A	09-04-1999	FR 2769321 A	09-04-1999
		AU 9355598 A	27-04-1999
		BR 9812845 A	08-08-2000
		CN 1278301 T	27-12-2000
		EP 1019510 A	19-07-2000
		WO 9918214 A	15-04-1999
		US 6143334 A	07-11-2000
US 5756103 A	26-05-1998	US 5494807 A	27-02-1996
		US 5762938 A	09-06-1998
		US 5759552 A	02-06-1998
		US 5756102 A	26-05-1998
		US 5766597 A	16-06-1998
		US 5863542 A	26-01-1999
		US 5766599 A	16-06-1998
		US 5989561 A	23-11-1999
		US 5759553 A	02-06-1998
		US 5744141 A	28-04-1998
		US 5843456 A	01-12-1998
		US 5997878 A	07-12-1999
		AU 701599 B	04-02-1999
		AU 1001197 A	15-05-1997
		AU 701781 B	04-02-1999
		AU 1001297 A	15-05-1997
		AU 672359 B	03-10-1996
		AU 1587192 A	06-10-1992
		AU 699903 B	17-12-1998
		AU 6051396 A	24-10-1996
		CA 2105277 A	08-09-1992
		EP 0575491 A	29-12-1993
		JP 6505874 T	07-07-1994
		KR 242671 B	02-03-2000
		US 5503834 A	02-04-1996
		WO 9215672 A	17-09-1992
		US 5833975 A	10-11-1998
		US 5641490 A	24-06-1997
		US 5766598 A	16-06-1998
		US 5942235 A	24-08-1999
		US 5759841 A	02-06-1998
		US 5514375 A	07-05-1996
		US 5364773 A	15-11-1994